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NAME OF SUPERVISOR/*NOM DU DIRECTEUR DE THÈSE* PEARSON K.G.

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THE UNIVERSITY OF ALBERTA

# REFLEX CONTROL OF CAT WALKING

by

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A THESIS
SUBMITTED TO THE FACULTY OF GRADUATE STUDIES AND RESEARCH
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FACULTY OF GRADUATE STUDIES AND RESEARCH

The undersigned certify that they have read, and recommend to the Faculty of Graduate Studies and Research, for acceptance, a thesis entitled Reflex Control of Cat Walking submitted by Jacques E. Duysens in partial fulfilment of the requirements for the degree of Doctor of Philosophy in Physiology.

KG Pearson
Supervisor

R B Stein

S M Padeh

Mary C. Wetzel
External Examiner

DATE October 26th 1976

> There are certain things in Nature in which beauty and utility, artistic and technical perfection, combine in some incomprehensible way: the web of a spider, the wing of a dragon-fly, the superbly streamlined body of the porpoise, and the movements of a cat. These last could not be lovlier even had they been designed by a preternaturally gifted dancer striving for choreographic grace, nor could they be more practical even under the tuition of that best of all 'coaches' - the struggle for existence. And it is almost as though the animal were aware of the beauty of its movements, for it appears to delight in them and to perform them for the sake of their own perfection.
>
> Konrad Lorenz

# ABSTRACT

The central motor program for walking is continuously under the influence of afferent input from receptors in the skin, muscles and joints activated during the walking movements. The way this afferent input reflexly modulates the step cycle of the single limb was investigated by activating these afferents during stepping and studying the induced changes in the EMG bursts or the joint angle excursions of either mesencephalic, premammillary or normal walking cats.

It was found that the reflex initiation of the swing phase does not only depend on a threshold hip extension, but rather is determined by reflexes from pad and foot receptors and from extensor muscles. Stimulation of the skin and muscle receptors either by natural stimulation methods (triceps surae stretch, vibration, evoked contractions) or electrical stimulation (weak shocks given to the skin of the distal hindlimb or to the nerves innervating this area) had a very potent inhibitory effect on the central system responsible for the generation of flexion during the swing phase (the flexor "half-center"). This suggests that initiation of flexion is inhibited by activity in skin and muscle afferents which are normally active when the limb is supporting the animal during stance, hence preventing the limb from flexing as long as the limb is loaded.

Termination of the swing phase is also likely to be under reflex control. Weak stimulation of the tibial nerve at the end of the flexion phase was found to shorten the period of flexion and to initiate

a premature onset of extension in premammillary cats. It was suggested that this reflex effect, presumably linked to activation of large afferents from the pad, forms the locomotory counterpart of the Hering-Breuer reflex, known to play a role in the termination of the inspiration phase. Further skin or skin-nerve stimulation experiments in the premammillary and normal cat revealed the existence of a second type of reflex response at the end of the flexion phase, this time, however, leading to a prolongation and exaggeration of the flexion movement. It was suggested that this response was related to the withdrawal reflex. During the stance, the evokation of exteroceptive extensor reflexes was found to increase the extensor activity, indicating that extensor reflexes may play a role in the maintenance of extension during the stance.

In conclusion, reflexes from exteroceptive and proprioceptive origin seem to fulfill two basic functions during cat locomotion:
(i) intensify the ongoing activity in a particular phase, and
(ii) determine the switching from one phase to another.

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# CHAPTER 1. INTRODUCTION

In mammals, alternating movements, such as seen in walking and respiration, are largely dependent on a centrally generated rhythm, which persists even in the absence of all sensory feedback (Brown, 1911; Burns and Salmoiraghi, 1960; Winterstein, 1911). Afferent input and reflexes are, nevertheless, essential for the normal execution of these cyclic movements, since the basic rhythm has to be adjusted to suit external requirements. Typically, the two phases within a cyclic movement differ in this respect. In a number of invertebrate and vertebrate cyclic motor systems, a distinction can be made between one phase, which is rather rigidly centrally programmed, and another phase, which is more flexible and relies more on sensory feedback. The inspiratory phase of respiration and the swing phase of locomotion are both characterized by a standard duration, set by a "central oscillator", while variations in total cycle duration mostly are brought about by reflexly induced changes in the duration of the expiratory phase and the stance phase (Gautier *et al.*, 1973; Orlovsky and Shik, 1965; Pearson and Duysens, 1976). However, even the more centrally dominated phases do not escape reflex control. In the mammalian respiratory system, artificial inflation of the lungs or stimulation of the vagus nerve during inspiration results in a premature termination of the inspiratory phase (Hering-Breuer reflex). When applied during the expiratory phase, the same maneuvers delay the expiratory-inspiratory phase transition.

As far as locomotion is concerned, the existence of phase limiting or prolonging reflexes is less well documented. In some invertebrates specialized receptors are thought to play a role in walking. When hair-plate receptors, which are normally excited during flexion movements, are removed in stick insects or cockroaches, exaggerated stepping with prolonged flexion is observed (Wendler, 1966; Wong and Pearson, 1976). In the mammalian walking system the reflex control of walking has been proposed repeatedly (Lundberg, 1969; Philippson, 1905) but direct evidence has been slow to emerge. Hence most of our present knowledge is still largely based on extrapolations from reflex studies on immobile animals and it is but recently that preparations have been developed allowing an experimental verification of these extrapolations (Shik *et al.*, 1966; Orlovsky, 1969).

## 1.1 *Neural Control of Locomotion*

Three recent reviews have covered this area extensively (Grillner, 1975; Shik and Orlovsky, 1976; Wetzel and Stuart, 1976). In general, one can distinguish the following basic elements in the organization of mammalian locomotion:

### 1.1.1 *The single limb oscillator*

Each limb has the ability of performing stepping movements on its own, even if the limb is deafferented (Brown, 1913, 1914; Shik and Orlovsky, 1965). During such stepping movements the limb acts as a whole so that disturbances in the movement of one joint produce a reaction in the movements of all joints (Orlovsky and Shik, 1965). The single limb oscillator is located in the spinal cord (Brown, 1913).

### 1.1.2 *Coupling between oscillators*

The use of more than one limb in locomotion requires the coordination of the stepping movements of different limbs. Such coordination is retained in spinal animals (Miller and Van Der Meché, 1976) but higher centers (brainstem and reticular formation) are thought to be involved as well ("tegmental response", Shik and Orlovsky, 1976). Afferentation is not indispensable for the production of coordinated stepping movements (Brown, 1911b) but is crucial for the adjustment of the different gait patterns (walk, trot, gallop) to the needs of the environment.

### 1.1.3 *Supraspinal centers*

The fine tuning of the "raw" progression movements is accomplished in higher vertebrates by the interplay of peripheral and central influences in supraspinal centers. These centers also determine when and how strong the spinal locomotory centers are activated (Shik and Orlovsky, 1976; Wetzel and Stuart, 1976).

## 1.2 *The Role of Afferent Input in Locomotion*

From the above it follows that afferent input is important at all levels of the organization of locomotory behavior. However, the work presented in this thesis has not been undertaken to gain more insight into the role of afferents on interlimb coordination or on higher center functioning. Instead, attention was entirely focused on the interaction between afferent input and single limb oscillator output. In particular, the question was asked how the step cycle and its different components are modulated by sensory influx. These step cycle components, conventionally designated as F, $E^1$, $E^2$, and $E^3$ (Philippson, 1905), can be briefly described as follows:

(i) *F (flexion phase)* is the period of the step between the lifting of the foot from the ground and the onset of extension in the major joints during the last part of the swing phase.

(ii) *$E^1$ (first extension phase)* is the period between the onset of extension and foot touchdown.

(iii) *$E^2$ (second extension phase)*: following touchdown the limb extends at the hip but yields at the knee, ankle and metatarsophalangeal joints.

(iv) *$E^3$ (third extension phase)* covers the time between the end of the yield and the thrust-off at the end of stance.

On the other hand, the afferent input can be classified in three major groups depending on the receptors of origin: (i) cutaneous input: sensory information derived from activation of skin receptors. During locomotion it will be mainly the skin receptors of the pad and foot that will be activated during the stance. (ii) muscle afferent input: the muscles contain specialized sense organs called spindles and tendon organs, both of which are known to be active during walking (Severin *et al.*, 1967).
(iii) joint afferent input: the role of joint receptors in locomotion is completely unknown but suggestions have been made that hip joint receptors signalling hip position may be important for the reflex

initiation of the swing phase (Grillner, 1975).

Recording, stimulation and deafferentation techniques were used as well as behavioral analysis to explore the contribution of these three groups of afferents to the reflex control of stepping in a single limb of the cat.

# CHAPTER 2. THE ROLE OF CUTANEOUS AFFERENTS IN LOCOMOTION

## 2.1 *Introduction*

In this chapter the role of exteroceptive reflexes will be investigated in some detail. Such reflexes fall into two groups:

(i) *Extensor reflexes:* are easily obtained in the resting animal (extensor thrust: Sherrington, 1906; positive supporting reaction: Magnus, 1926; "stützreaktion": Pritchard, 1926; toe extensor reflex: Engberg, 1964), and their discovery immediately gave rise to speculation about the use of these types of reflexes for the reinforcement of extension during the locomotory stance phase (Sherrington, 1906; Philippson, 1905). However, the latter idea failed to be proven, since denervation or anesthesia of the foot yielded very small deficits in the walking behavior of cats (Sherrington, 1910; Engberg, 1964). Sherrington concluded that cutaneous reflexes may contribute but are not indispensable to locomotion.

This view has stood up to the present day. Recently Grillner (1975) has pointed out that the negative evidence could be attributed to inadequate testing conditions and it is possible that deficits would be revealed if cats were allowed to walk on unpredictable surfaces. Also, it is conceivable that different afferent systems serve the same purpose and the proprioceptors of the limb may take over the role normally played jointly by exteroreceptors and proprioceptors. Therefore, selective activation of cutaneous receptors during locomotion may provide more information than can be obtained from denervation experiments. In a previous study by Forssberg *et al.* (1975) it was shown that stimulation of the dorsum of the foot during walking results in a phase dependent reflex reversal. It was found that stimulation during the swing phase enhanced the flexion of the stimulated hindlimb while stimulation during the stance phase evoked a shorter but more pronounced extension. No data are available on the stimulation of other skin areas during locomotion.

(ii) *Flexor reflexes:* also were thought to be related to locomotion. Sherrington (1910) noted that certain "non-noxious" stimuli evoke a reflex flexion in the limb and he concluded that this reflex flexion was the flexion phase of the step rather than the withdrawal reflex. Although considerable progress has been made over the years in the differentiation of afferents from different origins, it is at present still unknown which afferents play a role in the reflex control of the flexion phase as suggested by Sherrington. Our ignorance is mainly due to the exclusive use of motionless animals in the study of reflex effects of different types of afferents. Although such static studies proved extremely valuable for the description of a widespread system of afferents which reflexly excite flexor motoneurons ("Flexor Reflex Afferents" or FRA), they did not allow determination of whether these afferents made reflex connections with flexors because of their role in the reflex control of the flexion phase or mainly because of their protective function in the withdrawal reflex.

Actually both the flexor reflex and the flexion phase may well originate in the same center since the motor program as expressed by the pattern of contractions and relaxations of the different muscles in the limb is almost identical for both movements (Sherrington, 1910). The difference between the nociceptive withdrawal reflex and the locomotory flexion phase may then depend on the type of input this common center, henceforth called flexor "half-center" (Brown, 1914), receives both from central and peripheral sources. Small nociceptive afferent fibers may cause the flexor half-center to be active for a long period, hence producing a sustained withdrawal reflex, while larger afferents may reflexly excite the half-center to a smaller degree, hence producing the flexion phase of the step (except if the flexor half-center is disinhibited such as in the spinal animal, when the large afferents may also produce a sustained withdrawal reflex).

In the present study the function of flexor and extensor reflexes in locomotion will be explored using either acute or chronic experiments on walking cats. Stimulation of the skin or the skin nerves of the distal hindlimb will be employed to investigate the role of these reflexes in the regulation of the step cycle.

## 2.2 *Preparations and Methods*

### 2.2.1 *Preparations*

The present study was based on three types of preparation: normal, mesencephalic and premammillary cats. The latter two "acute" preparations can walk on a treadmill either spontaneously (premammillary cats) or after stimulation of a part of the brainstem (mesencephalic cats).

### A. *Mesencephalic cats.*

Stimulation of the mesencephalic locomotor region (MLR), corresponding to the cuneiform nucleus, evokes locomotion in mesencephalic cats put on a treadmill (Shik *et al.*, 1966). In total 13 mesencephalic cats were prepared in this study but only 8 responded satisfactorily to brainstem stimulation. Mesencephalic cats can adjust their gait to the *speed* of the treadmill even if MLR stimulation is kept constant. A stepwise increase of the speed of the belt from 0.5 m/sec to 1.5 m/sec resulted in a reduction of the duration of the hindlimb step cycle by 49%. Most of this reduction was due to a shortening of the extension phase since the duration of the EMG burst in the triceps surae fell by 69% while the duration of the burst of pretibial flexor activity fell only by 34%. The shortening of the extensor burst was to be expected in view of the results of Shik *et al.* (1966), who found that the support phase is shortened by increasing the speed of the treadmill belt in mesencephalic cats stimulated with constant strength. On the other hand, Shik *et al.* (1966) showed that there was a slight increase in the duration of the swing phase with increased speed. Since we found a shortening of the flexor burst with increased speed, it seems that the relative contribution of flexion and extension to the swing phase changes somewhat with changes in velocity. At higher speeds the swing phase presumably contains more extension and less flexion in the mesencephalic cat.

The role played by MLR stimulation in the *initiation and maintenance of walking* on a treadmill was illustrated by some interesting observations on mesencephalic cats which continued stepping for a short time despite discontinuation of the brainstem stimulation. Fig. 1 shows that stopping the stimulation of the MLR did not immediately stop the stepping, which instead could continue for several minutes. The duration and amplitude of the bursts of extensor activity in the triceps surae were drastically reduced after the termination of the stimulation, leaving only a short extensor burst during a period presumably corresponding to the first extension phase. Despite the dramatic changes in extensor EMG bursts the locomotory rhythm remained quite stable, the step cycle duration being reduced by only 8%.

These observations agree particularly well with the results of Severin *et al.* (1967) based on recordings of single ventral root filaments in mesencephalic cats. These Russian workers showed that brainstem stimulation selectively affects the number of motor units active at a given time while the locomotory rhythm is dictated by the speed of the belt and by the resulting afferent feedback rather than by brainstem stimulation. They found that an increase in MLR stimulation may lead to the recruitment of extensor motoneurons in the later parts of the support phase and that discontinuation of the MLR stimulation results in a gradual reduction in the duration of the rhythmic bursts of extensor activity.

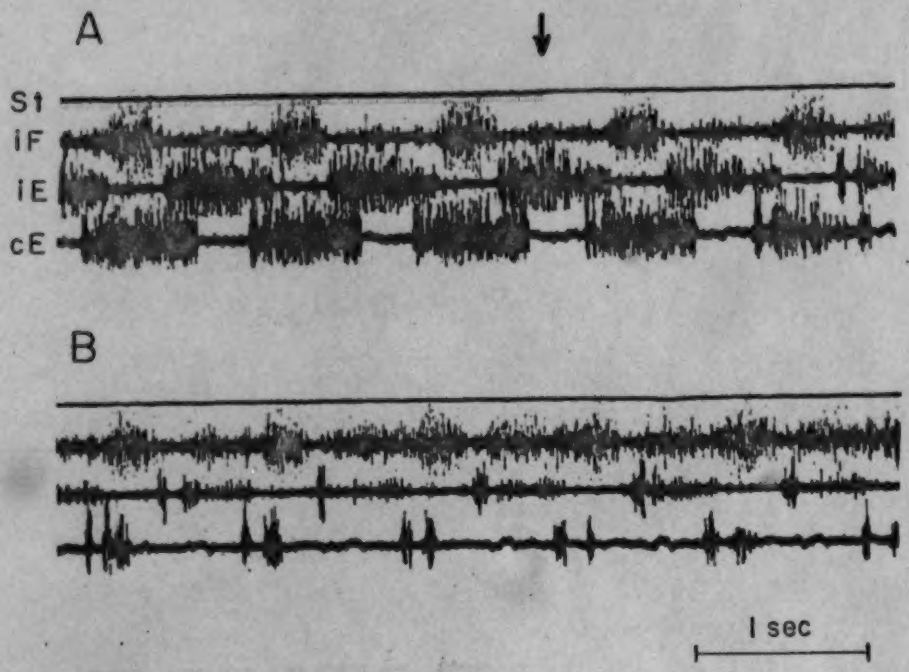


Fig. 1. Transition from evoked to spontaneous locomotion. Note the complete disappearance of the later parts of the extensor EMG burst after discontinuation of the brainstem stimulation (arrow). Lower part of figure is continuation of top part. St = stimulation of mesencephalic locomotor region. iF = unidentified hip flexor; iE = ipsilateral triceps surae; cE = contralateral triceps surae.

In addition to confirming the results of Severin *et al.* (1967), the present findings provide evidence that the first part of the extensor burst may differ fundamentally from later parts of extensor activity because of its relative independence of descending activity evoked by MLR stimulation. Since this first part of the extensor burst is likely to occur during the swing phase and is not affected by the termination of MLR stimulation, it seems that MLR stimulation selectively affects the extensor activity during the stance phase. The MLR stimulation could activate extensor motoneurons by way of direct descending pathways but it is hard to understand why such descending activity should only excite extensor motoneurons during the stance and not during the first extension phase. More likely, MLR stimulation "produces increased sensitivity of the spinal mechanisms to rhythmic afferent influences" (Shik and Orlovsky, 1976). In other words, MLR stimulation may increase the gain of afferent feedback pathways involved in maintaining the appropriate extensor activity during the stance. This gain adjustment could ensure that afferent input elicited from cutaneous receptors in the pad and from stretch receptors in the extensor muscles during the stance excites extensor motoneurons to a sufficient extent to support the animal.

### B. *Premammillary cats*

By making the decerebration in a slightly more frontal plane (just in front of the superior colliculus and the mammillary body), one obtains an acute "premammillary" cat (term introduced by Wetzel and Stuart, 1976) which is able to walk spontaneously without any brainstem stimulation. Usually the walking occurs in cyclic bursts, but sometimes premammillary cats can walk continuously for hours.

Apart from the technical advantage of not having to use brainstem stimulation, premammillary cats offer the opportunity to study rhythmic alternating locomotor output in a hindlimb which is partly denervated and immobilized (further details given in next section). Rhythmic contractions in the intact muscles of the immobilized hindlimb during stepping of the other free limbs occur readily in premammillary cats but more rarely in mesencephalic cats, presumably because premammillary cats usually walk with much more vigor than mesencephalic cats. Since fixation of one hindlimb in a walking cat allowed the application of a wide range of experimental procedures, it was decided to use predominantly the premammillary preparation for most of the experiments to be reported on.

### C. *Normal cats*

The use of decerebrate animals in locomotion studies may lead to interesting conclusions, but the extrapolations of these conclusions to the locomotory behavior of normal cats may have its dangers. Therefore, some of the experiments were done on normal cats which were trained to run on a treadmill. For this purpose the cats were deprived of food (for periods of less than 24 hours) and then put on the treadmill, on which they could run, in order to get a food reward. Some of these cats, belonging to another laboratory in our department, had multi-electrode neural cuffs implanted around several nerves (Stein *et al.*, 1975; Jhamandas, 1976), making it possible to use chronic recording and stimulation techniques in normal cats walking on a treadmill.

### 2.2.2 *Methods*

#### A. *Surgery (acute experiments)*

Experiments were done on adult cats weighing 2 - 4.5 kg. Until decerebration the animals were kept anesthetized with Halothane. After opening the skin, the common carotid artery was ligated bilaterally and on one side a cannula was inserted for blood pressure monitoring. Throughout the entire experiment the blood pressure was kept above 100 mm Hg by giving a 6% dextrose solution intravenously when the pressure dropped. A tracheotomy was performed, allowing the insertion of a T-shaped cannula in the trachea.

For the experiments with nerve stimulation, the left hindlimb was partially denervated at the hip (all branches of the obturator and femoral nerve) to reduce movements after fixation of the limb, and at the foot (peroneal, tibial and sural nerves) to reduce afferent input from the foot, which was later clamped for fixation. The partial denervation spared the hamstring muscles as well as the ankle flexors and extensors from which recordings could be made.

The animals were then transported to a frame over a treadmill. A stereotaxic headholder was used to fix the cat's head while the hip was clamped with two sharp hip bars. Xylocaine was used to anesthetize the insertion points of the hip bars. The left hindlimb was held up by two clamps, one around the knee and another around the foot. To avoid excessive pressure on tendons of long toe muscles, the foot clamp was not too rigid. Nevertheless, movement at the ankle was quite restricted and the contractions of the ankle flexors and extensors were essentially isometric. The knee was held in a semi-flexed position, allowing the construction of an oil pool. The three remaining limbs were in contact with the belt of the treadmill and were free to move.

Once fixed in the frame on the treadmill, the cats were decerebrated with a stereotaxically guided spatula. The level of transection was just frontal to the colliculi and the mammillary body in the case of the "premammillary" preparation and was just behind the mammillary body in the case of the "post-mammillary" or "mesencephalic" preparation. Both preparations can show good coordinated walking for several hours once the effects of the anesthetic wear off. The speed of the treadmill was kept at 2.7 km/hour. During walking, the innervated muscles in the fixed hindlimb contracted with normal timing, as if the limb were free to move.

In another group of cats, used for skin stimulation, the four limbs were left intact and a pair of silver plate electrodes (6 mm diameter) was attached to the shaved skin of the central plantar surface of the foot (interelectrode distance, 1 - 2 cm), or to the central and peripheral lobes of the hindlimb pad.

Finally, some premammillary cats had a neural cuff implanted around the sciatic nerve and at least one other cuff around the posterior tibial, common peroneal or sural nerve. These cats needed no denervation since the hindlimb containing the cuffs did not have to be fixed to allow the application of electrical stimuli.

### B. *Stimulation*

Square pulses from a digitimer and an isolation unit were used as stimuli. For stimulation of the skin, 50 - 600 msec trains of 1 msec shocks were used, while the nerve stimulation was done with 100 - 400 msec trains of 0.05 msec or 0.01 msec pulses. The frequency of pulses within each train was 60 Hz except for a few cases where 80 Hz was used. The stimulus trains were given at regular intervals of 4 sec or longer during the periods of walking. The interval was chosen such that the train was not synchronized with a particular time within the step cycle, but fell essentially at random within the cycle.

For the nerve stimulation experiments a threshold determination of the stimulated nerve was done in the resting animal prior to and following each period of walking. Single cathodal stimuli were applied to the nerve under study while the resulting compound action potential was recorded with the electrodes on the sciatic nerve. After threshold determination, the stimulus strength was increased until a maximum first peak was recorded.

The electrodes used for nerve stimulation in the fixed hindlimb consisted of a pair of platinum-iridium hook electrodes with an adjustable interelectrode distance of 0 - 15 mm. These electrodes were placed in a warm paraffin pool made in the knee cavity of the fixed hindlimb. The cuff electrodes, on the other hand, were placed in the free hindlimb. They were described in detail elsewhere (Stein *et al.*, 1975, 1976).

The following list gives an overview of the numbers and types of stimulation experiments used in this study.

- skin stimulation (acute) (N=8)
- nerve stimulation (acute)
  - (i) hook electrodes
    - tibial nerve (N=5)
    - posterior tibial nerve (N=3)
    - common peroneal nerve (N=5)
    - sural nerve (N=9)
    - cutaneous surae lateralis n. (N=3)
  - (ii) cuff electrodes
    - posterior tibial nerve (N=3)
    - common peroneal nerve (N=1)
- nerve stimulation (chronic)
  - posterior tibial nerve (N=2)
  - common peroneal nerve (N=1)
  - sural nerve (N=1)

Under "tibial nerve" is understood the tibial nerve at the popliteal fossa, while "posterior tibial nerve" refers to the same nerve at the ankle at a point distal to where the triceps surae nerve joins the tibial nerve. Most experiments were done with the nerves cut either at the knee or at the ankle and no systematic study was made of the differences in effect with the nerves left intact.

### C. *Recording*

In the *acute* experiments, the EMG activity of the ankle flexors and extensors was recorded bilaterally. A more precise identification of the recorded muscles was only possible in the fixed hindlimb where usually the lateral gastrocnemius (GL), the anterior tibial (TA) and sometimes the semimembranosus (SM) were selected. The electrodes for each muscle consisted of a pair of copper wires, insulated except for the tip, and inserted within 1 cm from each other in the muscle. The EMG signals were fed through a preamplifier and displayed on a four-channel oscilloscope, connected with a four-channel FM tape recorder.

In the *chronic* experiments on normal cats, the EMG of the triceps surae was recorded using the differential signal obtained from an internal and external lead of the cuff around the tibial or sural nerve. No flexor EMG was available but in some experiments, high speed cinematography (64 frames/sec) was used to verify that pauses between bursts of extensor EMG corresponded to flexion movements during the stepping. Matching of EMG records and film frames was achieved with the help of stimulus trains which were fed into a digital voltmeter and recorded on tape.

In all experiments the data were written out on a polygraph (Grass, model 7D) and/or filmed from the oscilloscope screen during or after the course of the experiments.

### D. *Data analysis*

In the *acute* experiments, very stable walking could be obtained so that some data could be analyzed by computer (Lab-8 computer system, Digital Equipment Co.). A program called "LOCO" was written by Dr. R.B. Stein to allow the automatic measurement of the duration of the EMG bursts preceding, during and following an applied stimulus. Fig. 2 illustrates an example of the analog readout of the data after they have been rectified and filtered. The short horizontal excursions at the beginning and ending of each EMG burst indicate the point at which a computer measurement was taken of the existing time difference with time "zero", the onset of the stimulus. The critical voltage level at which a measurement was taken was kept as standardized as possible, but sometimes adjustments had to be made when baseline irregularities approached the critical level too closely. A 7.8 msec bin width, combined with delays around 20 - 25 msec due to the filter time constant, resulted in a delay of approximately 30 msec. The digital printout of the data was compared with the original records to check for irregularities arising from stimulus artefacts and biological variability. Further quantitative treatment of the results, including a correction for the above mentioned 30 msec delay, was done with the help of some simple computer APL routines. The parameters were defined as follows (Fig. 2):

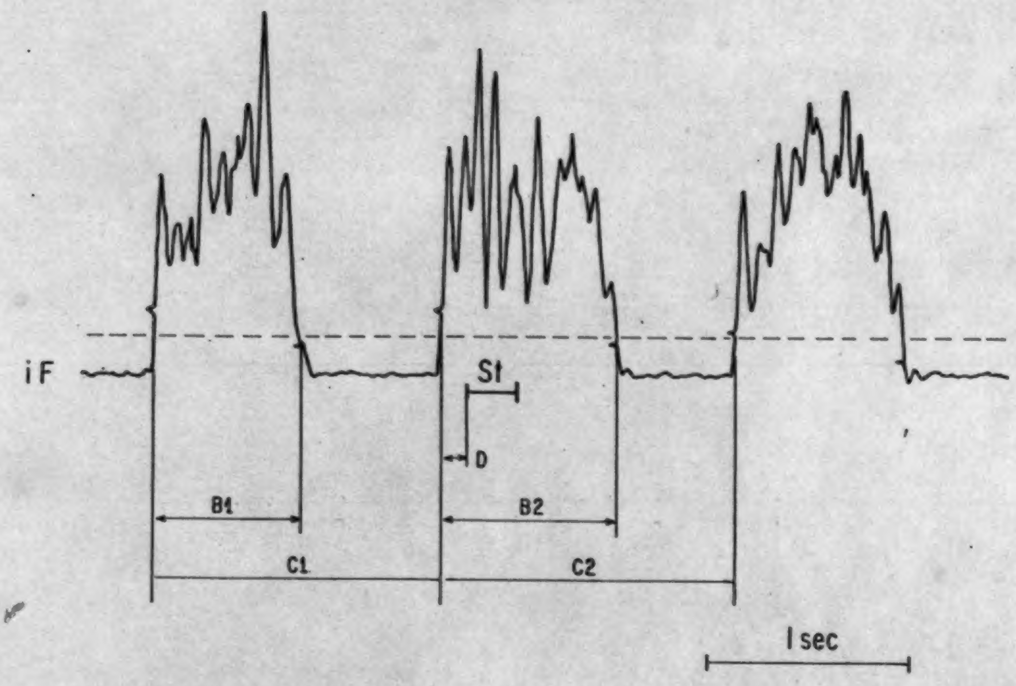


Fig. 2. Analog readout of rectified ipsilateral flexor EMG bursts (iF). Automatic time measurements are made at the points indicated by the small horizontal deflections occurring at the crossing of a preset critical voltage level (dashed horizontal line). The variability in the occurrence of the small horizontal deflections is due to the bin width of 7.8 msec. The figure represents an example of prolongation of the burst of activity in the pretibial flexors due to stimulation of the tibial nerve at 2.5 x T at the beginning of the flexion phase.

$C_1$: The control step cycle, preceding the step cycle with stimulation.

$C_2$: The step cycle during which stimulation was begun. Step cycle is defined as the time between the onset of two consecutive flexor bursts.

$B_1$: The duration of the control EMG burst preceding the EMG burst contained in $C_2$.

$B_2$: The duration of the EMG burst within the step cycle with stimulation ($C_2$).

D : Delay between onset of step cycle $C_2$ and onset of stimulus train.

The difference between the duration of the stimulated step cycle (or the EMG burst within this cycle) and the preceding control step cycle (or EMG burst) was calculated, normalized with respect to the control cycle (or burst) and expressed as a percentage:

$$\left( \frac{(C_2 - C_1)100}{C_1} \quad \text{and} \quad \frac{(B_2 - B_1)100}{B_1} \right) .$$

Similarly, the stimulus delay (D) was expressed as a percentage of $C_1$: ( $\frac{D \times 100}{C_1}$ ). The normalized differences were then plotted as a function of the normalized D.

In the *chronic* experiments on normal cats, there was of course much more variability in the step cycle parameters since these cats were not fixed in a frame over the treadmill. Moreover, stimulation often caused the normal cat to stop walking, to turn around, or to change the rhythm of stepping so that only short-term changes in the locomotory behavior could be studied. Therefore, the analysis on normal cats was limited to the effects produced on the duration of the phase in which stimulation was applied. To be able to make some kind of prediction about the duration which a particular phase would have had if no stimulation had been applied, only those stimulus trials were analyzed where the two equivalent phases preceding the stimulus application did not differ in duration by more than 60 msec (arbitrary criterion). In the example of Fig. 3, the stimulus train was started in the flexion phase (SF) of the stimulated hindlimb and the trial was accepted for analysis since the duration of the two preceding flexion phases (PPF and PF) did not differ by more than 60 msec. Usually less than one-third of all trials passed the 60 msec criterium.

The duration of the phase in which stimulation was started was compared to the duration of the preceding equivalent phase and the difference was expressed as a percentage of this preceding phase. The normalized differences were then plotted versus the time at which stimulation was started within the particular phase (DF or DE in Fig. 3). This time was calculated as a percentage of the preceding equivalent phase (DF/PF % or DE/PE %). For example, a stimulus started at a time when the flexion phase would be expected to be terminated on the basis of the duration of the preceding flexion phase would be a "100%" stimulus. Such "100%" stimuli could rarely be analyzed because the stimulus artefact in

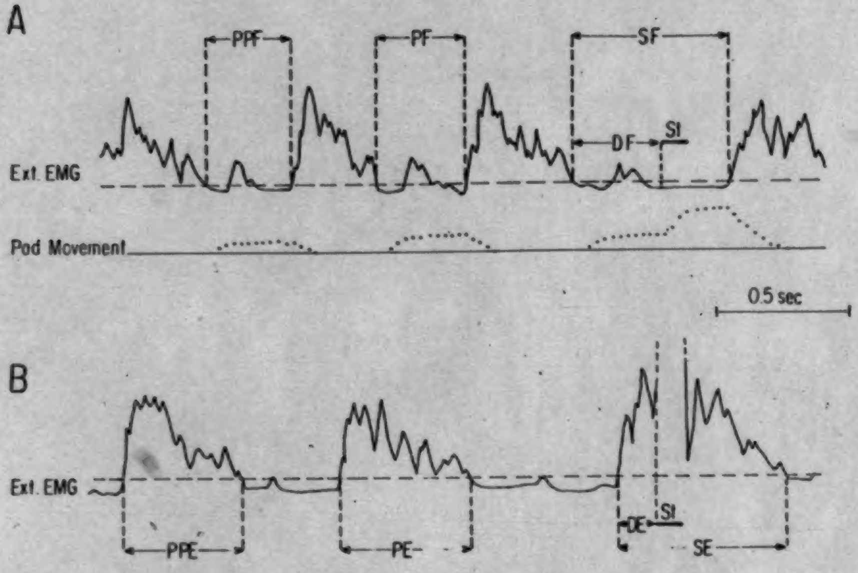


Fig. 3. Effect of stimulation of the posterior tibial nerve (2 x T) on the burst of EMG activity in the ankle extensors of the normal walking cat. (A) Stimulation during the pause between extensor EMG bursts causes the next burst to be delayed (SF > PF) due to extra flexion of the limb as seen from the filmed movements of the pad. In (B) stimulation is applied during the extensor EMG burst causing the total duration of the burst of extensor activity to be prolonged. Stimulus artefact eliminated from integrated EMG trace in A, while indicated by dashed line in B. Note the pick-up of flexor activity during the flexion phase, expecially in A. Further explanation in text.

the EMG trace often made it impossible to decide the exact onset of the following phase.

## 2.3 *Results*

### 2.3.1 *Skin stimulation*

#### A. *Plantar surface of the foot*

Weak electrical stimulation of the plantar surface of a thalamic cat under *resting* conditions evoked a small reflex contraction of the ipsilateral triceps. Stimuli which were 10 - 20 times stronger elicited a reflex flexion of the whole limb (flexor reflex). These two classes of stimuli, one producing an extensor, the other a flexor reflex, will from now on be classified as "weak" and "strong". The transition from an extensor reflex to a flexor reflex with increasing stimulus intensities has been described by Sherrington (1904) and Sherrington and Sowton (1911).

(1) *The weak stimuli*, a 300 msec train of 1 msec pulses at 60 Hz, were then applied at regular intervals during the periods of spontaneous walking. Since no attempt was made to synchronize all stimulus trains with a particular phase of the step cycle, application of the stimuli was essentially at random within the step cycle.

When the weak stimuli fell within the stance phase of the ipsilateral hindlimb, a marked effect on the ipsilateral extensor activity was observed (Fig. 4). Both amplitude and duration of the ipsilateral triceps EMG burst were increased. There was also a delay in the onset of the subsequent activity in the ipsilateral flexors and the contralateral extensors. Taken together, these

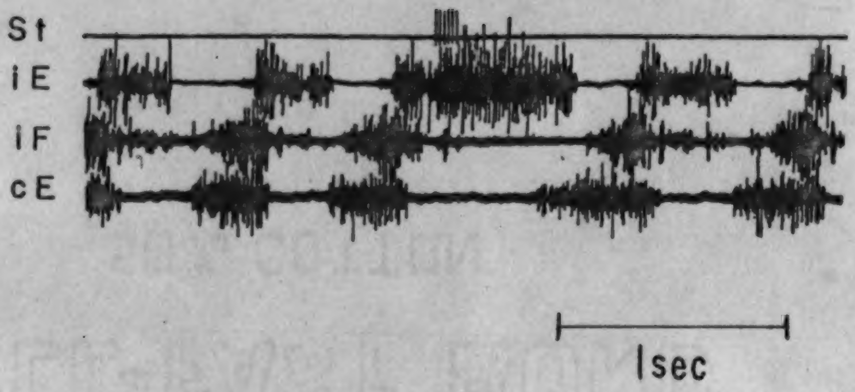


Fig. 4. The increase in amplitude and duration of extensor activity following stimulation of the central plantar surface of the foot during the stance phase of a premammillary cat walking on a treadmill. A short train of six weak electrical shocks (St., 1 msec, 60 Hz) given immediately after the first extension phase evokes a long and intense discharge in the ipsilateral ankle extensors (iE). The prolonged extension is associated with a delay in the onset of the next EMG burst of the ipsilateral pretibial flexors (iF) and the contralateral ankle extensors (cE).

flexors and the contralateral extensors. Taken together, these changes produced a bilateral prolongation of the step cycle, defined as the sum of a flexor and an extensor EMG burst. The situation was quite different when weak stimuli were applied during the swing phase. This is illustrated in Fig. 5, obtained from another experiment. Fig. 5A shows how stimuli given at the beginning of the ipsilateral flexor activity prolonged the flexor EMG burst while having no appreciable effect on extensor activity. Thus, the over-all effect is again a prolongation of the step cycle, but this time it is the longer flexor burst which is responsible for the step cycle prolongation. In between is the situation with weak stimulation starting in the middle of the swing phase (Fig. 5B). The stimulus train, extending over the end of the flexor and the beginning of the extensor burst, caused a prolongation of the ipsilateral flexor activity while at the same time reducing the duration and the amplitude of the ipsilateral extensor burst. The shortening of the extensor activity caused a shortening of the step cycle. The three situations with stimulation at the beginning (Fig. 5A), middle (Fig. 5B) and end (Fig. 5C) of the step cycle are further described in Fig. 5D. The duration of the step cycle during which stimulation was started was compared to the duration of the just-preceding step cycle and this difference was plotted against the interval between the onset of the cycle and the onset of the stimulus train (see Section 2.2). Thus, data points lying above the horizontal zero axis indicate a prolongation of the step cycle while the lower points indicate a shortening.

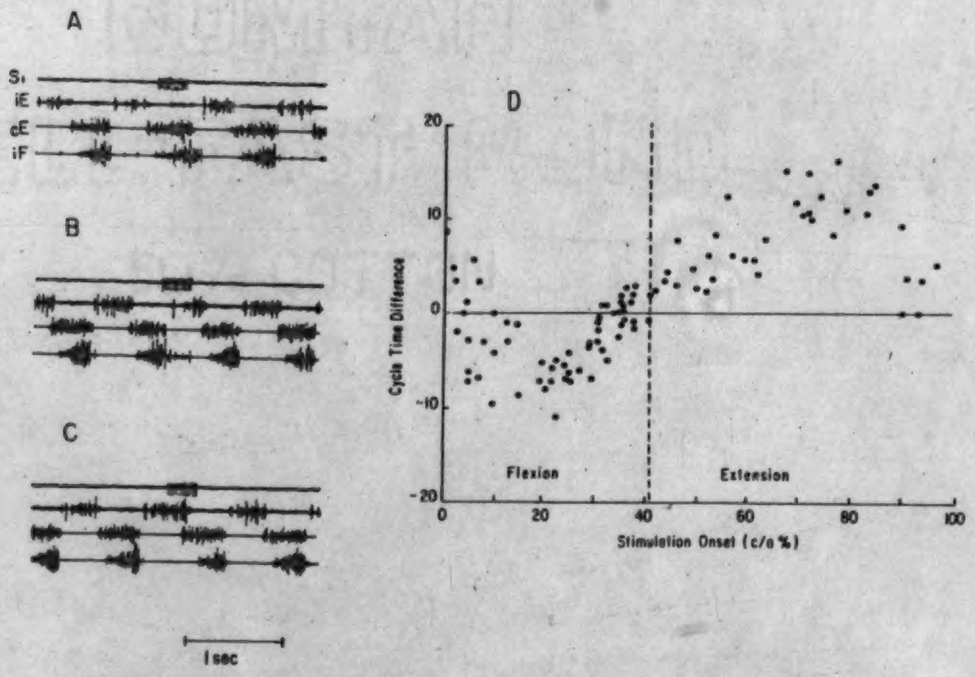
Fig. 5. Step cycle changes evoked by electrical stimulation of the central plantar foot at different moments of the ipsilateral step cycle. Different cat from Fig. 4.

A. A train of shocks given at the onset of the ipsilateral pretibial flexor activity (iF) prolongs the flexor burst. The following ipsilateral extensor burst (iE) is of normal duration but the total step cycle duration (flexor + extensor burst) is prolonged.

B. The same stimuli given at a later moment in the step cycle slightly prolongs the ipsilateral flexor burst and at the same time reduces the ipsilateral extensor activity both in amplitude and duration. Note the resulting shortening of the step cycle.

C. Stimulation of the plantar surface of the foot during the period of activity in the ipsilateral triceps surae prolongs the ipsilateral extension and delays the onset of the next flexion. The result is a prolongation of the step cycle.

D. Scatter diagram illustrating the changes in the step cycle duration for a large number of consecutive sequences such as seen in A, B and C. The abscissa represents the time of onset of stimulation within the step cycle (D in Fig. 2). The ordinate gives the difference in duration of the step cycle ($C_2$ - $C_1$ in Fig. 2). All data are expressed as a percentage of the step cycle preceding stimulation (control cycle $C_1$). The dashed vertical line separates the period of activity of the pretibial flexors (on the average 41% of the control cycles) from the period of activity of the ankle extensors. Notice the prolongation of the step cycle when the stimulus train starts either early in the phase of flexor activity or during the period of extensor activity (see A and C). A shortening of the step cycle is seen when stimulation is started in the middle of the flexor activity (see B). Stimulus parameters: square pulses of 1 msec, 60 Hz, in trains of 300 msec at intervals of 3 - 5 sec. The stimuli were subthreshold for the evocation of a flexion reflex in the resting animal.


A
S₁
iE
cE
iF
B
C
1 sec
D
20
10
0
-10
-20
Cycle Time Difference
Flexion
Extension
0
20
40
60
80
100
Stimulation Onset (c/a %)

On the average, the step cycle just preceding the cycle with stimulation had a duration of 787 msec (S.D. = 23 msec or 2.9%). Flexor EMG activity accounted on the average for the first 41% of this time (dotted dashed line in Fig. 5). The effects on step cycle duration described for Figs. 5A, B and C are readily recognized in Fig. 5D. Step cycle prolongations are noted when stimulation starts either early in the swing phase or in the middle of the stance phase. A pulse train given in the middle of the swing phase caused a shortening of the step cycle. Again, as for Fig. 4, the lengthening of the ipsilateral step cycles was correlated with a lengthening of the contralateral cycles. In the stimulated limb there was a clear tendency for the prolonged or shortened step cycles to be followed respectively by a shorter or longer step cycle, indicating a return to the control rate of stepping.

(ii) *The strong stimuli* produced, as mentioned above, a flexor reflex in the resting animal (Fig. 6A). Interestingly, however, the late flexor component of the flexor reflex often formed the first event in a series of steps (Fig. 6B). This suggests that these two phenomena, the late flexor reflex component and the locomotory flexor activity, have much in common and, in fact, may arise from the same spinal center (see Section 2.1).

Fig. 6B also illustrates another common finding, namely that the late flexor reflex component was often preceded by a small amount of triceps surae activity. This "concealed" extensor reflex (Creed *et al.*, 1932) could, at times when walking was not

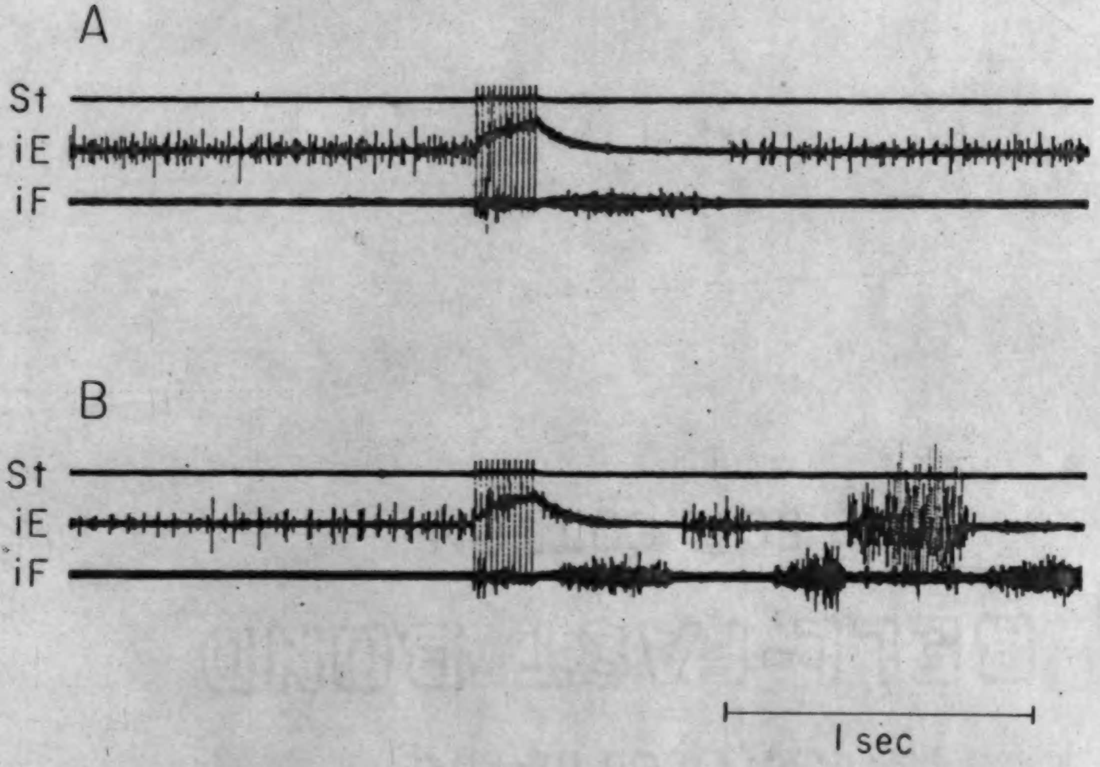


Fig. 6. Stimulation of the plantar surface of the foot evoking a flexor reflex with an early and a late component in the resting animal (A) and in the animal starting to walk (B). Note similarity between the late flexor reflex component and the rhythmic flexor bursts. In (B) a "concealed" extensor reflex is seen just after termination of the stimulus train (St = 200 msec train of 0.5 msec pulses at 60 Hz). iE = ipsilateral triceps surae; iF = ipsilateral prebitial flexors. Large stimulus artefact yielded a DC shift in the iE trace.

very forceful, become so prominent as to completely over-shadow the late flexor reflex component, which then failed to appear. The "conversion" of a flexor reflex into an extensor spasm could occasionally occur in the resting animal, but was much more frequent in the walking animal, as was also observed by Lisin *et al.* (1973).

### B. *Pad*

#### (1) *Weak stimuli*

Similar results were obtained when the pad was stimulated instead of the plantar surface of the foot. *At rest*, weak pulses (60 Hz) applied to the plantar cushion for a period of 400 msec evoked a toe extensor reflex (Philippson, 1905; Engberg, 1964). Slightly stronger stimuli evoked ankle extension, indicating a transition from the toe extensor reflex to the extensor thrust (Sherrington, 1910). Finally, very strong stimuli evoked a flexion of the ipsilateral hindlimb (flexor reflex: Sherrington, 1910).

Weak stimuli were now applied during *walking*, producing much the same results as in the case of the plantar foot stimulation. Stimulation starting during the period of activity of the ipsilateral flexors resulted in a shortening of the step cycle of up to 20% (Fig. 7A, C), while stimulation starting during the activity in the ipsilateral extensors gave cycle prolongations of up to 30% (Fig. 7B, C). The larger scatter in Fig. 7C is probably linked to the more irregular walking during the period analyzed for Fig. 7C, as compared to the period used for Fig. 5. In fact, the variability

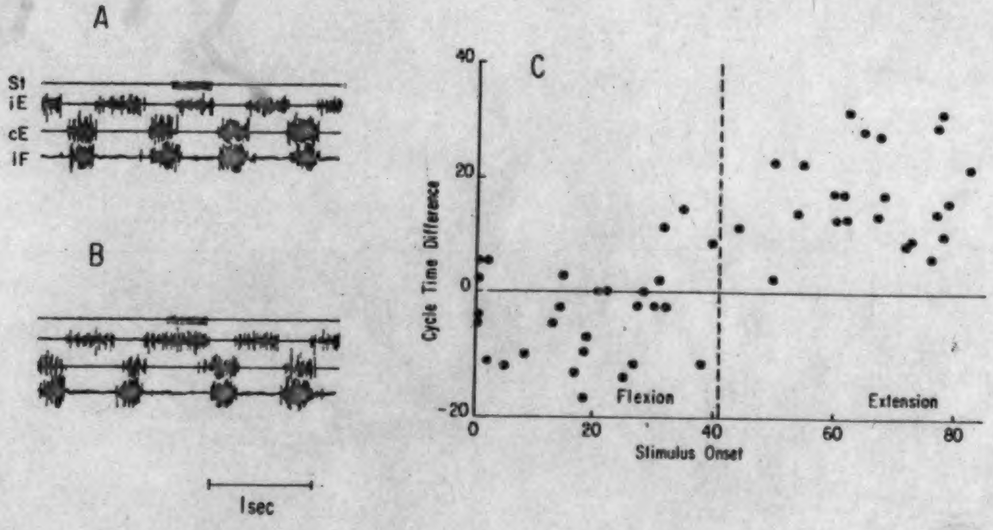


Fig. 7. Effect of electrical stimulation of the pad at different moments in the step cycle.

A. Pad stimulation starting during the flexor burst (iF) has an inhibitory effect on the amplitude and duration of the activity of the ipsilateral triceps surae (iE). The shortening of the step cycle is due to the short extensor burst while the flexor burst is unchanged.

B. Stimulation of the pad during the ipsilateral stance phase. The ipsilateral extensor activity is prolonged and the next flexor burst is delayed, causing a prolongation of the step cycle.

C. The changes in step cycle duration observed in A and B are plotted for a large sample of consecutive step cycles. Abscissa and ordinate as in Fig. 5D. Stimulation starting in the period of flexor activity causes a shortening of the step cycle while stimuli applied during the extensor activity prolong the step cycle. Stimulus parameters are the same as for Fig. 5D but duration of pulse train is 400 msec.

of the control cycles for Fig. 7C was much larger (S.D. = 51.7 msec or 7.4%, average cycle duration = 702 msec).

#### (2) *Strong stimuli*

A slight increase in the stimulus strength caused a weak flexor reflex in the resting animal. During walking the stimuli had much the same effect as before, evoking flexor burst prolongations (Fig. 8A) and extensor burst prolongations, but in addition a new phenomenon was observed. As illustrated in Fig. 8B, some of the stimulus trains given during the flexion phase caused a brief flexor burst interruption followed by a resumption of the flexor activity. A response of this type corresponds to what Sherrington (1913) called "rebound" and consequently this term will be used henceforth to designate a response which consists of a brief inhibition of ongoing activity followed by an increased comeback of this activity. The flexor rebound activity was often remarkably similar to the rhythmically occurring flexor burst not only with respect to its duration but also with respect to its shape as characterized by a gradual build-up of EMG activity.

### 2.3.2 *Nerve stimulation*

#### A. *Hook electrodes*

In the previous section it was assumed that mostly cutaneous receptors would be stimulated by the electrical shocks.

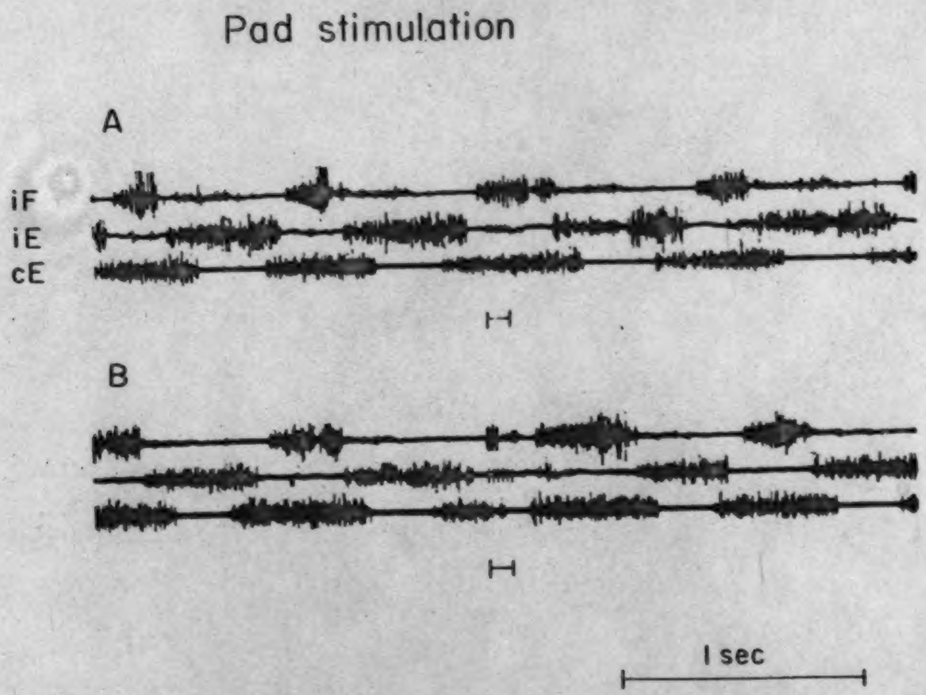


Fig. 8. The effect of electrical stimulation of the pad on the locomotory output of the intact hindlimb. Flexor burst prolongation (A) or rebound (B) was associated with prolongation, with (B) or without (A) interruption of the contralateral extension phase. Note the short duration of the flexion phase. Stimulus: 100 msec train of 1 msec pulses at 60 Hz. Symbols as in Fig. 4. Figure retouched to reduce stimulus artefact.

However, the possibility that underlying muscle was also activated could not be excluded. Therefore it was important to see if the previous results could be repeated by stimulating cutaneous nerves directly. Nerve stimulation has the added advantage that a better control can be exerted over the type of afferents stimulated (since the diameter of a nerve fiber is inversely related to its electrical excitability threshold).

In order to be able to directly stimulate hindlimb nerves during locomotion, it was necessary to fix the hindlimb containing the stimulated nerves (see methods). This manoeuver did not prevent the rhythmic activation of the flexors and extensors in the fixed hindlimb, but it introduced some changes in the locomotory output. The burst of pretibial flexor activity which normally takes up only about one-third of the step cycle duration now could occupy more than 50% of the total cycle duration. This was mainly due to the ankle being fixed in an extended position or to the triceps surae being denervated, since proprioceptive input from the ankle extensors plays an important role in the determination of the duration of EMG burst in the pretibial flexors (see next chapter). The artificial prolongation of the flexor burst was actually more of an advantage than a disadvantage since it allowed for the demonstration of certain reflex effects which are very hard to demonstrate with other techniques.

### (1) *Sural nerve*

#### (a) *Weak stimulation*

There are several reasons for starting with the study of the sural nerve. Firstly, the sural nerve is considered a pure cutaneous nerve, while cutaneous afferents from the pad join mixed nerves (tibial nerve). Secondly, the sural nerve innervates the skin of the lateral part of the plantar foot (Hunt and McIntyre, 1960) and this makes a comparison possible with results from the first part of this study. Thirdly, with respect to the extensor reinforcing reflexes seen after skin stimulation during the stance phase, it is of particular interest that there exists a well defined pathway from the low threshold sural nerve afferents to ankle extensor motoneurons (Lloyd, 1943; Wilson, 1963). Moreover, the skin areas innervated by the sural nerve (lateral plantar surface of the foot, ankle, skin overlying the triceps surae) are likely to be activated during certain forms of locomotion such as walking in a crouched position.

*At rest*, weak tetanic sural nerve stimulation evoked a reflex contraction of the ipsilateral isolated triceps surae with a delay of 25 msec or less. This is in agreement with previous work by Hagbarth and Naess (1950), who showed that there is a facilitation of the ankle extensors after 30 msec of repetitive stimulation of the sural nerve. To determine the type of afferent fibers involved in this extensor reflex, L7 dorsal root recordings were made in some animals. The afferent volley, evoked by threshold stimulation of the sural nerve, reached the dorsal root with a latency of 2.5 -

3 msec, indicating that fibers with a conduction velocity of 73 - 88 m/sec were activated. Such fibers are amongst the largest found in the sural nerve and they are presumably involved in the transmission of information about touch and pressure (Hunt and McIntyre, 1960; Whitehorn *et al.*, 1974).

Trains of weak stimuli were then applied during the periods of *walking* (Fig. 9). When given during the swing phase, the stimuli tended to reduce the amplitude and duration of the next extensor burst, causing a slight reduction in cycle duration. The most striking effect, however, was seen when stimulation was delivered during the period of extensor activity. The pulse train both enhanced and prolonged the extensor activity and caused a prolongation of the cycle. Sometimes the prolongation was more marked than can be seen in Fig. 9, since several points fell completely beyond the scale of the ordinate of the graph. Such examples are illustrated in Fig. 10. A stimulus train given near the end of the stance phase prolonged the step cycle for almost 100%. This appeared to be due to deletion of one period of relaxation. In all cases where an ipsilateral flexor EMG was obtained it was noted that the flexor activity was completely suppressed during the prolonged step cycles. Moreover, omission of extensor relaxation occurred simultaneously with a complete (Fig. 10A) or partial (Fig. 10B) disappearance of the contralateral extensor activity.

These effects did not depend on the triceps surae nerve being intact or not. Fig. 11B shows how stimulation of the sural nerve at 2.5 x T increased the duration of the step cycle when

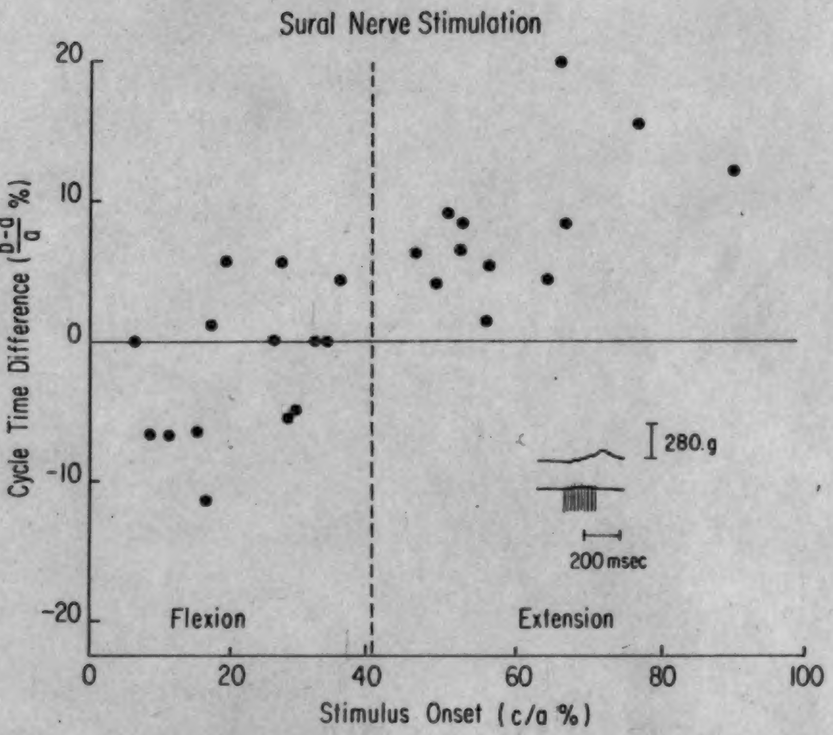


Fig. 9. Influence of weak tetanic sural nerve stimulation on the rhythmic contractions of ipsilateral ankle extensors and flexors during locomotion. Coordinates as in Fig. 5. The ipsilateral hindlimb being fixed, the "step cycle" duration is taken as the time between the onset of consecutive EMG bursts in the fixed pretibial flexors. Sural nerve stimulation starting during the contraction phase of the isolated triceps surae prolongs the triceps surae contraction and thus also the step cycle. Stimulation at an earlier time in the step cycle tends to shorten the step cycle. A 180 msec train of 1 msec pulses was used at 60 Hz. In the inset this same stimulus given to a resting animal is shown to produce a reflex contraction of the isolated triceps surae with a latency of 30 msec. An example of very pronounced step cycle prolongation falling completely beyond the scale of the ordinate of this graph is illustrated in Fig. 10B.

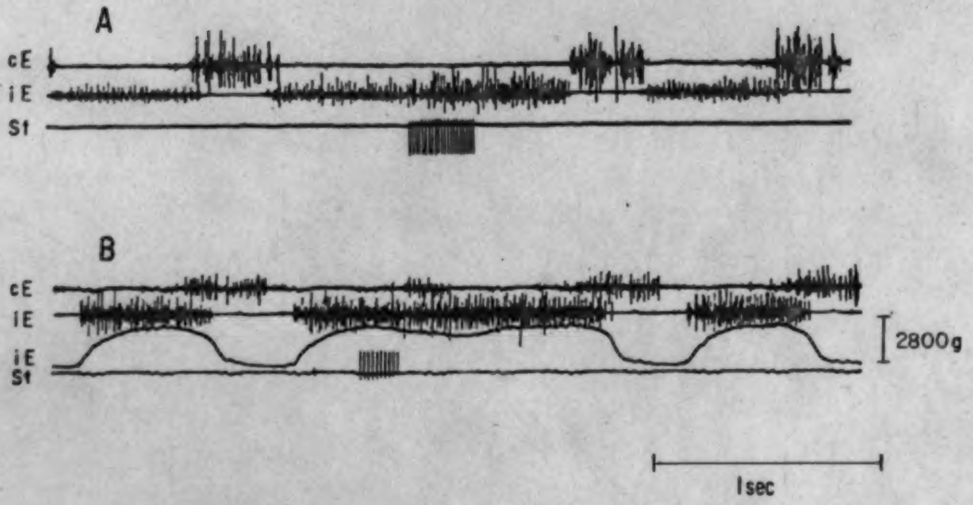


Fig. 10. Repetitive stimulation of the sural nerve at the end of the stance phase prevents the rhythmic relaxation of the ipsilateral triceps surae (iE). Concomitant with the prolongation of the ipsilateral extensor contraction there is a complete (A) or partial (B) inhibition of the contralateral extensor EMG (cE). For (A) a stimulus train of 280 msec (80 Hz) was used. In (B) 60 Hz stimulation was given for 150 msec. The stimuli shown in (A) and (B) evoked an extensor reflex in the resting animal. (A) and (B) are from different cats but (B) is from the same cat as in Fig. 9. Force calibration is for the third trace in (B), showing the force exerted by the ipsilateral triceps surae (iE).

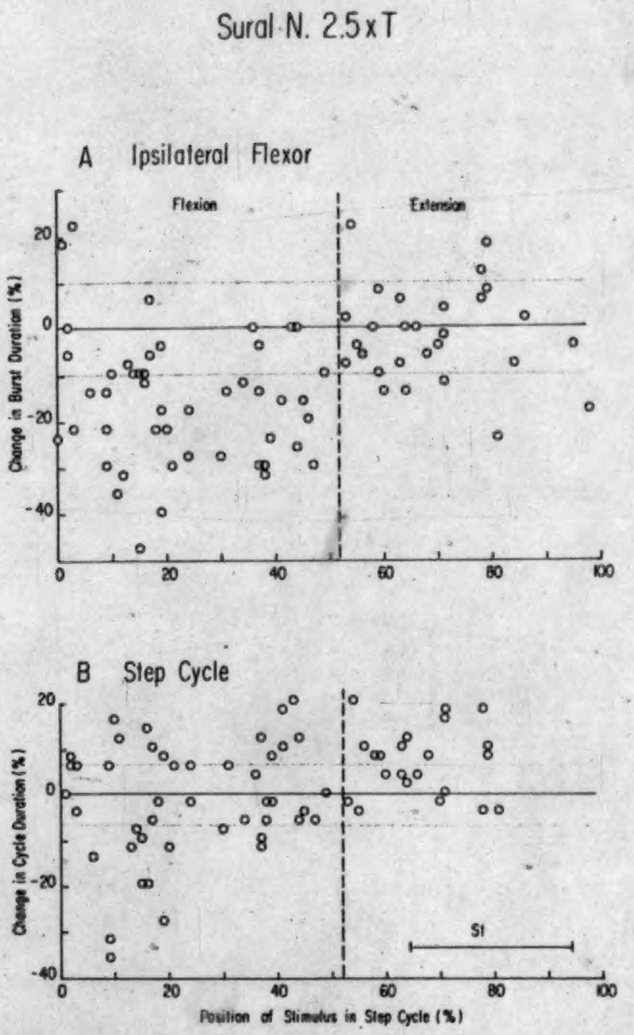


Fig. 11. Effect of sural nerve stimulation on the duration of the ipsilateral flexor burst (A) and on the step cycle duration (B). Tibial nerve cut proximal to triceps surae nerve. St = 300 msec, 60 Hz, 0.05 msec pulses. Data points referring to stimulation in the last 15% of the step cycle are omitted in B because the stimulus artefact made it impossible to determine reliably the onset of the flexor activity.

applied during the extension phase of a cat with the triceps surae nerve cut. The effects on the duration of the flexor burst are illustrated in Fig. 11A. Clearly, the 2.5 x T stimuli caused a shortening of the activity in the pretibial flexors. However, long trains (300 msec) were needed to demonstrate the flexor burst inhibitory effects and even then exceptions were noted since stimulation starting at the very beginning of the flexor burst failed to shorten the duration of the flexor activity and, in fact, prolonged it at times (Fig. 11A). The flexor burst prolonging effects for stimuli starting in the beginning of the flexion phase were even more pronounced when shorter trains of stimuli (100 msec) were used.

### (b) *Strong stimulation*

When describing the results of strong stimulation of the plantar surface of the foot it was suggested that the late flexor reflex component and the locomotory flexor burst may arise from activity in a common center, tentatively called flexor "half-center" (Brown, 1914). If the latter suggestion is correct then it should be of interest to see what happens when this common flexor half-center is activated from different sources at the same time. Such a situation can be created by evoking a flexor reflex during locomotion. Locomotion provides a natural source of activation of the flexor half-center while periodic stimulation of the sural nerve with strong stimuli (75 x T) evokes a flexor reflex, hence providing a second (but artificial) way of activating the flexor half-center.

Theoretically one could expect two types of effects on the locomotory rhythm when combining artificial and natural ways of activating the flexor half-center. If the flexor half-center formed merely an output stage of the stepping oscillator, present in each limb (see Chapter 1), then its artificial activation should not have much effect on the locomotory rhythm. On the other hand, if the flexor half-center is closely coupled or identical to the rhythm generating structure in each limb, then one would expect that the artificial activation of the flexor half-center would lead to a resetting of the rhythm in case such activation would not coincide with the natural rhythmic activation during locomotion.

To elicit a flexor reflex the sural nerve had to be stimulated by 7.5 x T. Fig. 12 illustrates how stimulation at 10 times this value (75 x T) affected the locomotory rhythm. Application of the stimulus during the occurrence of the ipsilateral burst of activity in the pretibial flexors prolonged the activity in these flexors so drastically as to cause a partial or total disappearance of the pause between two rhythmic flexor bursts (Fig. 12A). Often this made it difficult to decide whether one was dealing with one or two step cycles. Therefore, the effects of stimulation on the locomotory rhythm could better be judged by examining the contralateral side, where there was no ambiguity about the occurrence of a step cycle since the stimuli only caused a mild reduction of the amplitude and/or duration of the triceps surae activity. Effects of stimulation on the contralateral step cycle duration, measured as the time between the onset of consecutive extensor bursts, were

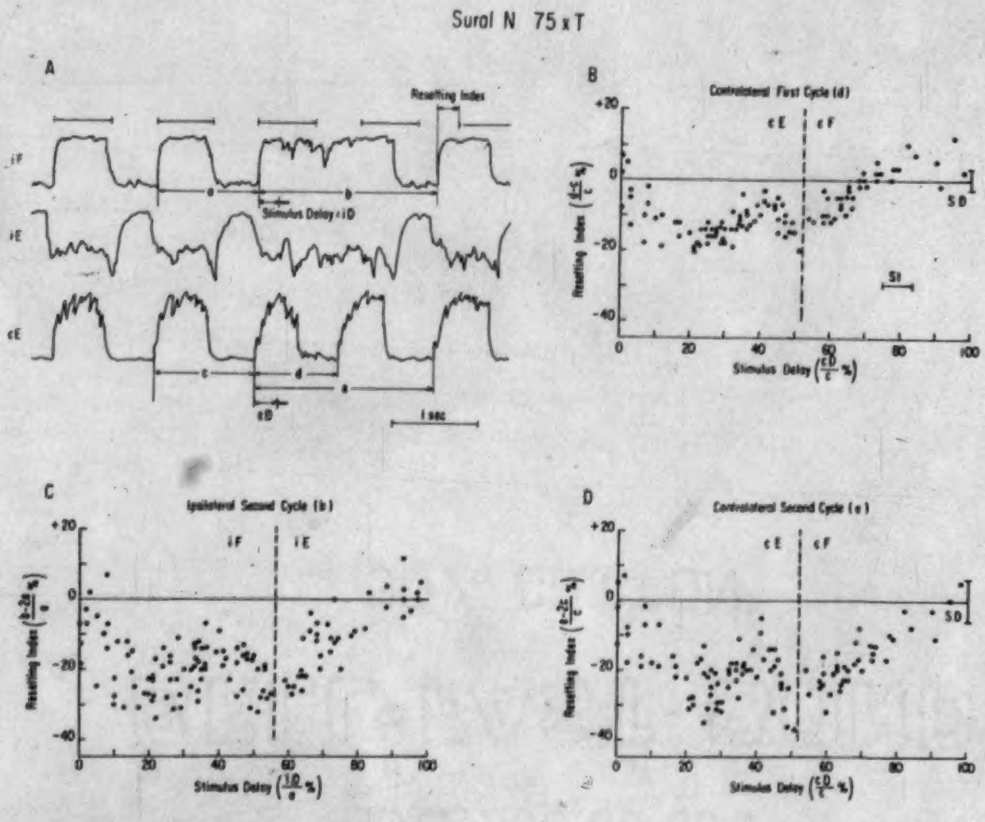


Fig. 12. The effect of sural nerve stimulation at 7 5 x T on the stepping rhythm. A: Example of stimulus trial. Stimulus delay (iD or cD) indicates time between onset of ipsilateral flexor burst (iF) or contralateral extensor burst (cE) and the onset of stimulus train (100 msec train of 0.05 msec pulses at 60 Hz). Bars on top of iF indicate expected occurrences of flexor bursts, while "resetting index" indicates difference between expected and actual onset of flexor bursts. B, C and D: Plots of resetting index for contralateral and ipsilateral step cycles. SD = variability of control cycle durations. St = duration of stimulus train expressed as a per cent of average control cycle. Further description in text.

limited to two step cycles: the step cycle during which stimulation was applied (first cycle or "d" in Fig. 12) and the next cycle (second cycle, ending at the end of "e"). The duration of the third cycle (starting at the end of "e") differed on the average less than 0.2% from the control duration ("c"). The induced changes in the duration of the first contralateral cycle were highly dependent on the time of stimulus application within the step cycle (stimulus delay, cD). As shown in Fig. 12B, most stimuli caused a shortening of the first cycle with respect to the control ("c"), but stimulus trains started at the beginning or at about 40% of the cycle yielded much less shortening while stimuli given at the end of the cycle actually caused a cycle prolongation. The effect on the second cycle was mostly a shortening effect so that a plot of the changes produced in the total duration of the first and second cycle ("e") showed an even greater tendency in the direction of shortening than the plot of the first cycle (Fig. 12D versus Fig. 12B). Since stimulation did not affect the duration of cycles following the second cycle, it follows that plots of the changes in the total duration of the first 3 or 4 cycles would essentially be identical to Fig. 12D except for larger scatter due to increased variability. For this reason, and also because Fig. 12D compares the total duration of the first and second cycle with the expected duration of these two cycles (prediction based on taking twice the duration of the step cycle preceding stimulation), one can read Fig. 12D as a plot of the resetting of the contralateral rhythm.

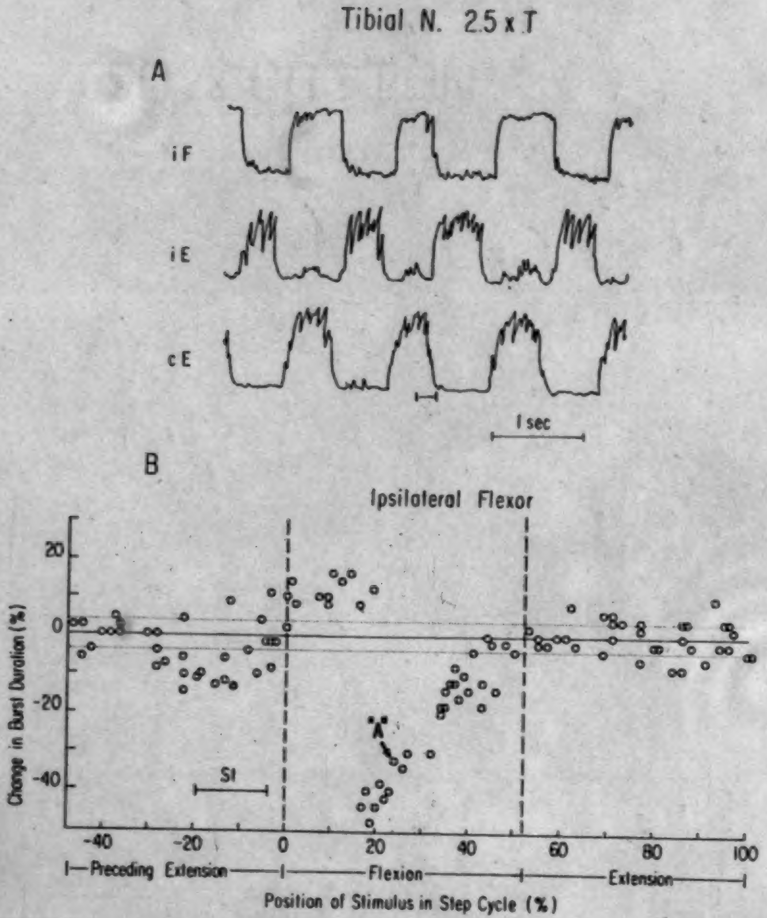
Ipsilaterally, the onset of the burst of activity in the pretibial flexors was closely correlated with the onset of the contralateral extensor activity. This made it possible to estimate which was the end of the second ipsilateral cycle corresponding with the end of the second contralateral cycle in cases where such decision was difficult to make solely on the basis of ipsilateral records (Fig. 12A). The plot of the changes in the total duration of the estimated first two ipsilateral cycles ("b") versus the time of stimulus application ("iD") was very similar to the corresponding contralateral plot (Figs. 12C and D) indicating a strong coupling between ipsi- and contralateral rhythms. Minimum resetting effects were observed when the flexor reflex was evoked just prior to or at the beginning of the ipsilateral flexion phase.

### (2) *Tibial nerve*

#### (a) *Weak stimulation* (up to 2.5 x T)

Low intensity stimulation of the tibial nerve at the knee (proximal to the triceps surae nerve junction) produced a prolongation of the extensor burst if the stimuli were applied during the extension phase. If applied at the end of the flexion phase the same stimuli induced an early termination of the flexor EMG burst and a premature onset of the extensor burst, which had a prolonged duration (Fig. 13A). The silencing of the EMG of the ipsilateral pretibial flexors occurred with a short latency of 35 - 60 msec, making involvement of supraspinal loops possible. The premature onset of the extensor activity was observed for both the semimembranosus

Fig. 13. Changes in duration of the burst of activity in the pretibial flexors due to 2.5 x T stimulation of the tibial nerve at different times in the step cycle. Polygraph example in A illustrates how a stimulus train (horizontal bar = 200 msec, 12 pulses of 0.05 msec at 60 Hz) given in the middle of the flexion phase causes the flexor burst to end earlier than expected and the extensor burst to start prematurely. iF = ipsilateral pretibial flexors; iE = ipsilateral semimembranosus; cE = contralateral triceps surae. The activity in iE during the flexion phase is due to pick-up of electrical signals from the nearby semitendinosus. The tibial nerve was cut proximal to the point where the triceps surae nerve joins the tibial nerve. However, the results were identical if recordings were made of the triceps surae instead of the semimembranosus in experiments where the tibial nerve was cut at the ankle, leaving the innervation of the triceps surae intact. In B a large number of trials such as seen in A were plotted according to the time of stimulus application in the step cycle (x axis represents the time between the beginning of the step cycle and the onset of stimulation). The durations of the flexor bursts are seen to be shortened (lower half of plot) for stimuli given during the last part of the flexion phase (arrow "A" represents the example given at the top of the figure). The variability of the flexor burst durations can be evaluated from the data points on the right side of the plot ("extension") since these points represent the durations of the flexor burst in step cycles where the stimulation was given after the occurrence of the flexor burst. In addition, a measure of the variability is given by the two horizontal dotted lines which are 1 SD removed from the zero horizontal line (SD calculated from flexor burst durations in step cycles without any stimulation). The bar marked "St" represents the duration of the stimulus trains as a percentage of the average control step cycle. Average duration of flexion phase = 618 msec or 52% of the duration of the control step cycle (= 1179 msec).


Tibial N. 2.5 x T
A
iF
iE
cE
1 sec
B
Ipsilateral Flexor
Change in Burst Duration (%)
20
0
-20
-40
St
"A"
-40
-20
0
20
40
60
80
100
Preceding Extension
Flexion
Extension
Position of Stimulus in Step Cycle (%)

and the triceps surae. The latter observation seems rather surprising since, to our knowledge, there are no reports of reflex activation of the semimembranosus by activation of tibial nerve afferents. However, it should be kept in mind that our knowledge of reflex connections is largely restricted by the use of immobile animals. The walking cat differs from these immobile preparations in that during locomotion the limb acts as a whole and thus one can expect that reflex actions may be much more widespread so as to involve the activation of particular spinal coordinating centers ("half-centers", Graham Brown, 1914).

The suppression of the flexor EMG was, however, restricted to stimuli given *after* the first quarter of the flexion phase. Stimulation applied during the first quarter of the flexion phase yielded a prolongation of the flexor burst instead (Fig. 2; Fig. 13B upper data points).

The timing of the breakpoint between evoked flexor burst prolongations and shortenings depended on the stimulus strength. For weaker stimuli (1.6 x T) shortening of the flexor burst only occurred in the period following the first 32% of the step cycle, compared with 15% when the stimulus strength was 2.5 x T. The lowest threshold encountered for flexor burst shortening was 1.17 x T (5 cats).

The prolonged or shortened flexor bursts were followed by shortened or lengthened extensor bursts respectively (Fig. 14A). As a result, the duration of the step cycles showed relatively small changes as compared to the large changes in the flexor burst duration

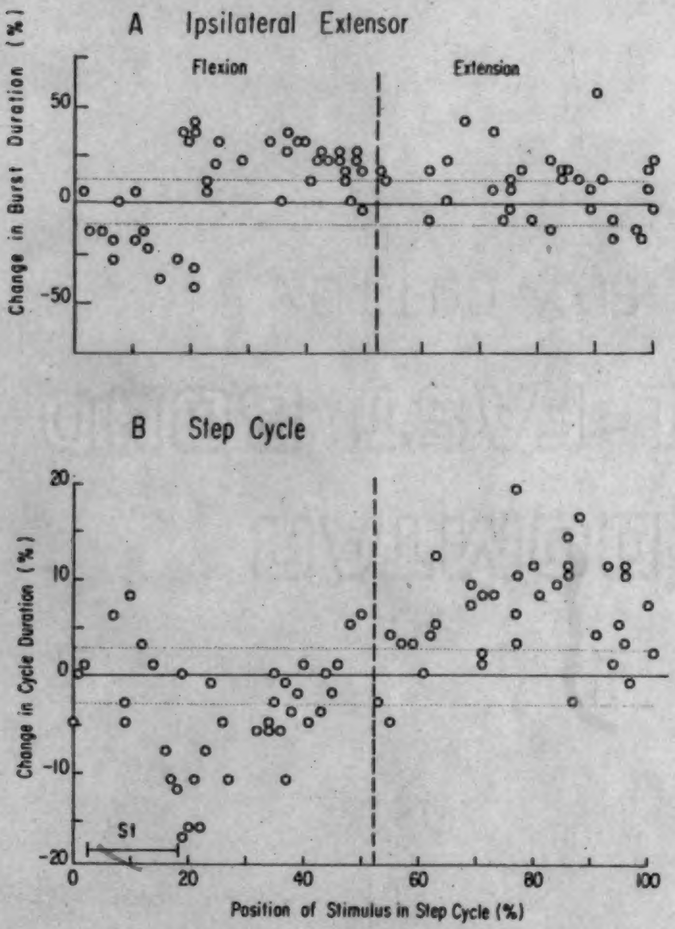


Fig. 14. Same cat and same sequence as Fig. 13, but analyzed for changes in duration of the ipsilateral extension (iE) and of the step cycle. Notice that stimuli applied during the flexion phase have either a shortening or prolonging effect on the following extensor burst. Stimulus same as in Fig. 13.

(Fig. 14B versus Fig. 13B). Nevertheless, there was a tendency for step cycle prolongations with stimuli given in the beginning of the flexion phase and for step cycle shortenings with stimuli given in the middle of the flexion phase. As expected from aforementioned results on pad stimulation, a prolongation of the step cycle was noticed for tibial nerve stimuli given during the extension phase (Fig. 14B). On the contralateral side a shortening of the extensor burst occurred in conjunction with a shortening of the ipsilateral flexor burst (Fig. 13A).

Since the tibial nerve contains afferents from the pad, one can ask whether the flexor burst rebound, observed after pad stimulation during the flexion phase (Fig. 8B) could also be seen after tibial nerve stimulation. Fig. 15A shows that this was indeed the case. In the experiments of Fig. 15A the flexor burst rebound did not always occur when stimuli at 2.3 x T were applied at about the same time in other step cycles. Instead the stimuli could evoke a premature ending of the flexor burst and an early onset of extensor activity as described in Fig. 13A. However, no responses were observed representing a transition between the flexor burst rebound response and the flexor burst shortening response. This strongly suggests that the mechanism underlying the generation of the first part of the flexor burst operates in an all-or-none fashion.

The question arises whether the flexor burst prolongations observed with tibial nerve stimulation at the beginning of the flexor burst (Fig. 2) are fundamentally different from the flexor burst rebound described in Fig. 15A. Rebound usually arises in a

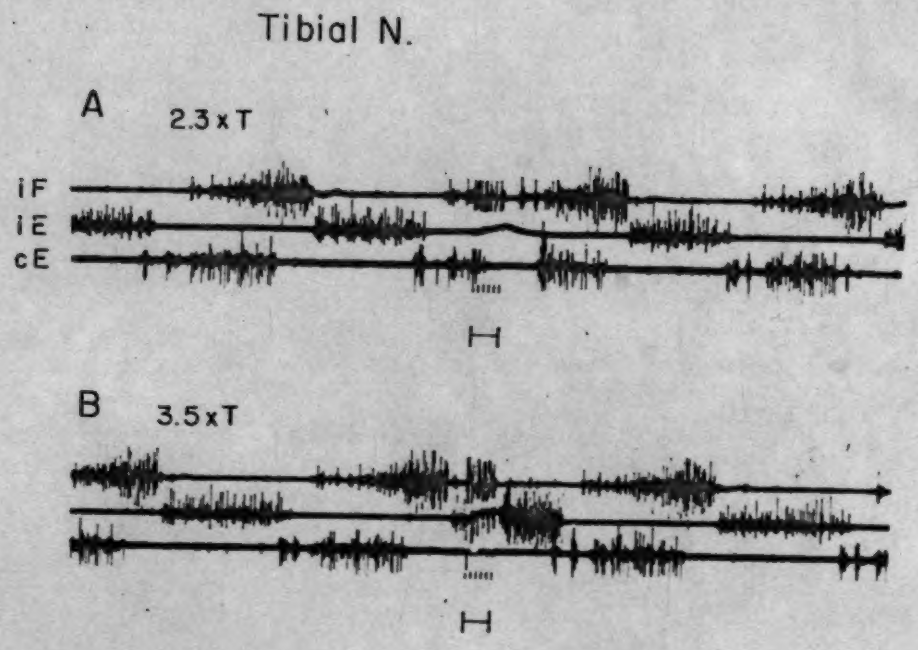


Fig. 15. Rebound of flexor (A) and extensor (B) activity due to stimulation of the tibial nerve during the flexion (A) or extension (B) phase. iF = pretibial flexors; iE = ipsilateral triceps surae; cE = contralateral triceps surae. Note the characteristic built-up shape of the normal and rebound flexor burst while the rebound burst of activity in the triceps surae does not resemble the normal extensor burst. Stimulation: 100 msec train of six stimuli, each 0.05 msec, at 60 Hz. Tibial nerve cut at ankle, leaving triceps surae innervation intact. Ankle fixed in extended position.

contracting muscle after an inhibitory stimulus has caused some initial relaxation. However, Brown (1911) and Sherrington (1913) have both described examples of flexor "rebound" occurring in the absence of flexor relaxation during the stimulus application. The presently observed flexor burst prolongations may be the result of a similar type of "rebound" with concealed inhibition but the question then arises whether one can still use the term "rebound" in this respect (see definition on page 33).

### ( b ) *Strong stimulation* (3.5 x T)

Rebound phenomena were not limited to flexors and to stimulation during the flexion phase but were also observed in the triceps surae when the tibial nerve was stimulated during the extension period (Fig. 15B). However, the triceps surae rebound differed from the rebound in the pretibial flexors in that much higher stimulus intensities (3.5 x T) were needed for the triceps surae rebound. In contrast to the flexor burst rebound which caused the total duration of the flexor burst to be prolonged, the triceps surae rebound was correlated with a shortening of the total duration of the extensor burst. Clear evidence for rebound was only obtained for the triceps surae and not for the semimembranosus.

The finding of triceps surae burst rebound suggested that at 3.5 x T afferents were recruited which were inhibitory to the extensor burst generating system. Further support for the existence of such inhibitory connections came from the observation that stimuli at 3.5 x T given during the flexion phase caused the coming extensor burst to be shorter or to disappear altogether. This reduction in extensor activity was associated with an increase in flexor activity. Hence, as illustrated in Fig. 16A (bottom), stimulation at 3.3 x T at the end of the flexion phase caused the space between two flexor bursts to be occupied by reflex flexor activity, replacing the absent extensor burst and creating an abnormally long following flexor burst. This type of prolonged flexor burst was first seen for 3.3 x T stimuli given at the breakpoint between evoked flexor burst prolongations and evoked flexor burst shortenings (Fig. 16B).

Further increases in stimulus strength accentuated the tendency towards suppression of the extensor burst and prolongation of the flexor burst until a point was reached where long sustained flexor responses completely interrupted the locomotory output.

### (3) *Posterior tibial nerve*

By stimulating the tibial nerve at the knee one cannot rule out that muscle afferents from the triceps surae contributed to the observed effects. Therefore, the posterior tibial nerve at the ankle was stimulated under the same conditions to see if any differences in results could be detected.

Stimulation of the posterior tibial nerve (2 x T) yielded a reflex activation of the ipsilateral triceps surae with a latency of 31 - 57 msec, resulting in a premature onset of the triceps surae activity when the stimuli were given at the end of the flexion phase. Rebound was seen both in the pretibial flexors and in the triceps surae (the semimembranosus was not recorded from in these experiments).

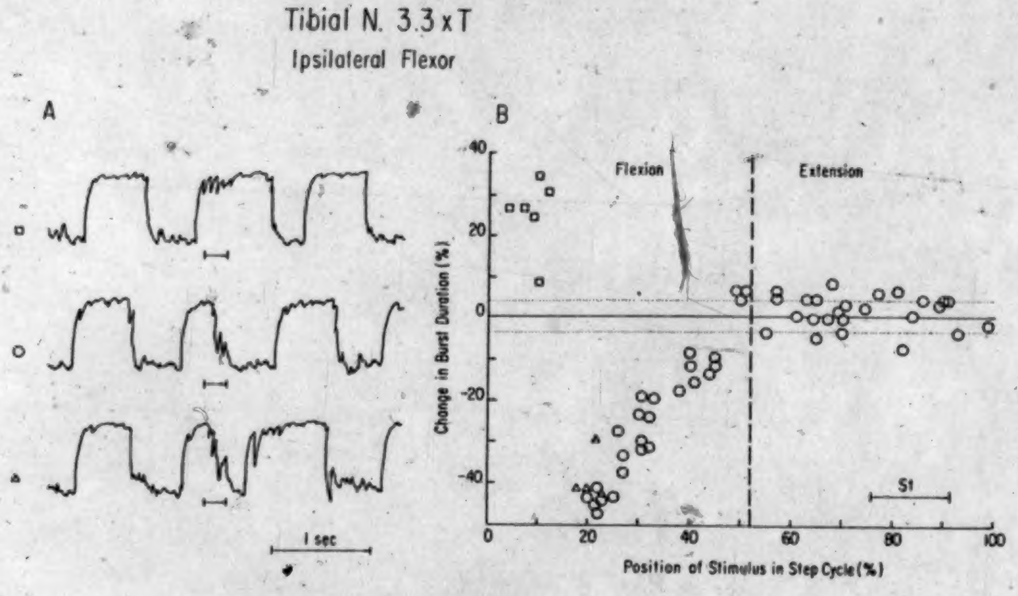


Fig. 16. Effect of tibial nerve stimulation at 3.3 x T on the duration of the ipsilateral flexor burst. Stimuli given in the beginning of the flexion period caused a flexor burst prolongation (□) while stimuli delivered at a later time in the flexion period evoked a flexor burst shortening (○ in A). The third type of response (△) was caused by the elimination of the normally occurring relaxation period between flexor bursts. The absence of flexor relaxation in the third type of response was correlated with the absence of an extensor burst. The occurrence of these three types of responses was related to the time interval between the onset of the step cycle and the onset of stimulation (B). Same cat as in Figs. 13 and 14.

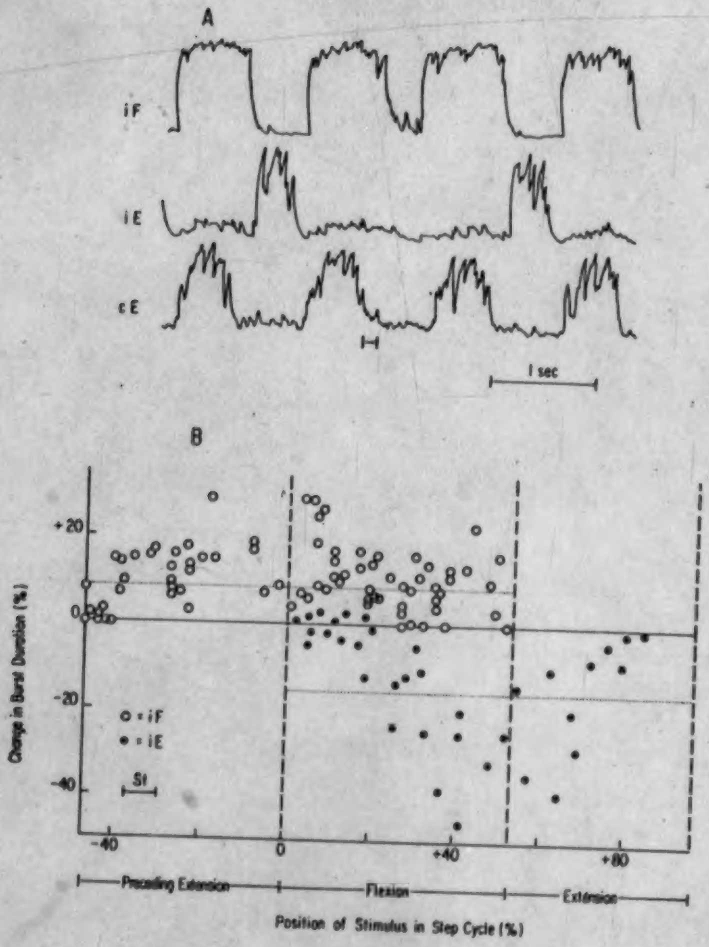
It thus seems that the effects obtained with tibial nerve stimulation at the knee are largely due to the activation of afferents already present in the posterior tibial nerve before the junction of the triceps surae nerves.

### (4) *Common peroneal nerve*

Although the common peroneal nerve probably contains afferents producing an extensor reflex (see section on chronic cats in this chapter), it was clear that most of the fibers in this nerve had flexor burst prolonging and extensor burst shortening effects when stimulated in the fixed hindlimb during locomotion. In the example given in Fig. 17A, stimulation of the common peroneal at 1.7 x T at the end of the flexion phase is seen to inhibit completely the following burst of activity in the semimembranosus. Coinciding with the absence of an extensor burst, a reduction in the relaxation period between flexor bursts is seen which induces the impression that the flexor burst following stimulation occurs at an earlier time than expected. This response was identical to the response described in Fig. 16A (bottom) for tibial nerve stimulation at 3.3 x T. The flexor burst prolonging and extensor burst shortening effects of common peroneal stimulation are further illustrated for a large number of stimulation trials in Fig. 17B taken from an experiment in which the extensor burst suppression was less dramatic than in the example of Fig. 17A. Shortening of the duration of the extensor burst resulted from stimulation during or before the extension phase. In contrast, stimulation during or before the

Figure 17. Effect of stimulation of the common peroneal nerve at 1.7 x T on the step cycle. In A stimulation was given at the end of the flexion phase, resulting in a complete suppression of the following EMG burst in the extensors (iE = ipsilateral semimembranosus). The absence of an extensor burst led to the premature onset of the next flexor burst (iF = ipsilateral pretibial flexors), while stepping at the contralateral side was not affected (cE = contralateral triceps surae). In B, taken from another experiment with less drastic extensor burst suppressing effects, the stimulation of the common peroneal nerve (1.7 x T) is shown to result in changes in the duration of the flexor and extensor bursts (iF, iE), depending on the time of stimulus application. The effects on the flexor burst were examined for stimuli given before or during the flexor burst while the effects on the extensor burst were studied for stimuli given before or during the extensor burst. The "1 SD" line above the zero horizontal line is for the flexor bursts, while the "1 SD" line below is for the extensor bursts. St = 100 msec train of 0.05 msec pulses at 60 Hz.

Common Peroneal N. 1.7 x T



flexion phase caused a flexor burst prolongation. Despite the pronounced changes in burst duration, there was only minimal disturbance of the locomotory rhythm. Prolongations of the flexor burst due to stimulation during the flexion phase were linked to shortening of the following extension phase. Similarly, shortening of the extensor burst due to stimulation during the extension phase was linked to prolongation of the coming flexor burst. In either case, a change in duration in one phase was partly compensated for by an opposite change in the duration of the next phase and the result was that the stepping rhythm was minimally perturbed.

### (5) *Cutaneous surae lateralis nerve*

This small cutaneous nerve perforates the biceps muscle before innervating the lateral side of the knee. Its electrical stimulation during locomotion yielded results which were quite similar to the results described for the common peroneal nerve. Stimulation (4.4 x T) before or during the extension phase shortened the duration of the extensor burst (Fig. 18A) while stimulation applied before (Fig. 18B) or during (Fig. 18C) the flexion phase prolonged the duration of the flexor burst. These changes were correlated with a shortening of the step cycle, especially when stimuli were given near the transition from flexion to extension (Fig. 18D).

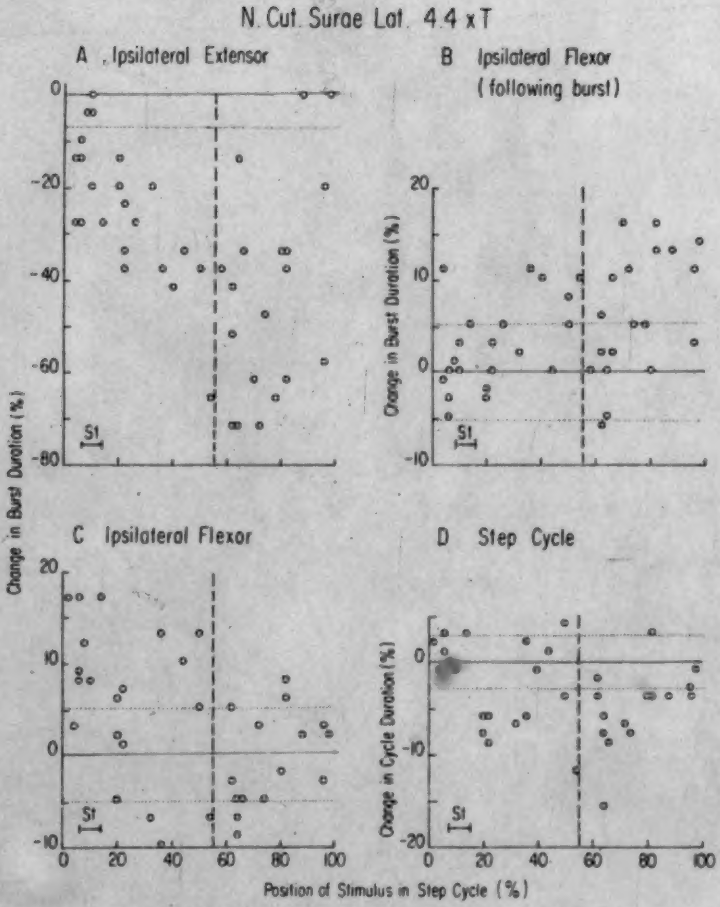


Fig. 18. Effect of stimulation of the cutaneous surae lateralis nerve on the duration of the extensor and flexor bursts during locomotion. In (B) the changes in the duration of the flexor burst following stimulation were plotted while (C) shows the changes in the duration of the flexor burst during the step cycle in which stimulation was started.

### (6) *Flexibility in the structure of the step cycle*

The results on weak stimulation of the plantar surface of the foot, the pad and the nerves innervating this area in the premammillary cat indicate that the locomotory system tends to keep the stepping rhythm constant despite large flexibility in the durations of the different phases of the step cycle. Evoked prolongations in one phase could occur at the expense of the duration of another phase without necessarily interfering with the duration of the total step cycle. This flexibility was further highlighted by some occasional observations of spontaneous disappearances of extensor bursts in the fixed hindlimb in animals which showed weak stepping of the right hindlimb and which had the left tibial nerve cut. Typically the absent extensor burst was followed by a flexor burst which was on the average 39% longer than the control flexor burst (N=18; Fig. 19A, B). The prolonged flexor burst was preceded by a shorter flexor silence, in association with the absent extensor burst. When the prolonged flexor burst duration was added to the duration of the short silent phase, the resulting "step cycle" differed only 2% from the preceding control "step cycle", indicating that the absence of the extensor burst had very little effect on the locomotory rhythm. Note that here the term "step cycle" exceptionally refers to a flexor relaxation and contraction, where previously "step cycle" indicated a flexor burst plus the following extensor burst.

## B. *Cuff electrodes*

The hook electrodes have the disadvantage that the hindlimb

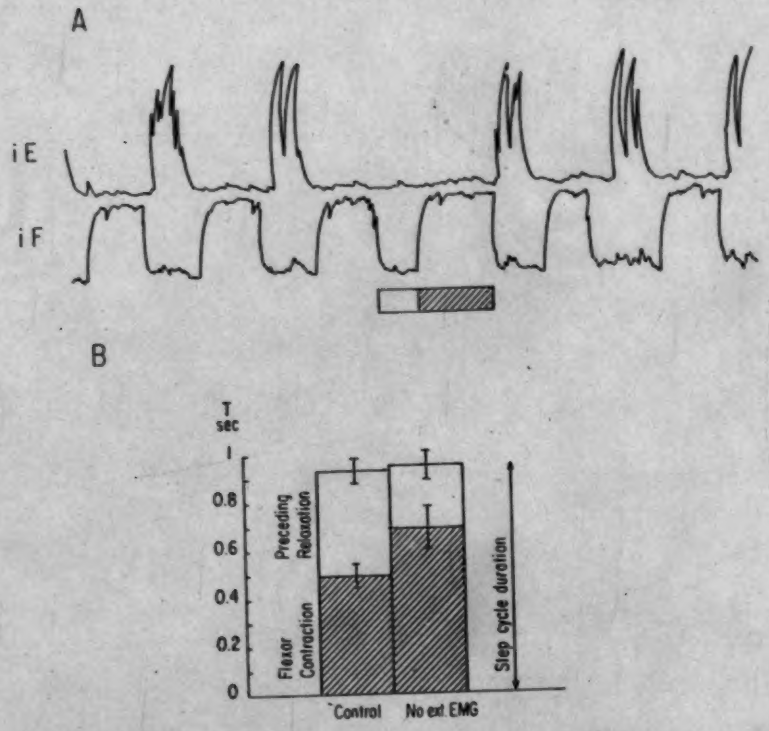


Fig. 19. Spontaneous disappearance of EMG burst in semimembranosus from the fixed hindlimb of a walking premammillary cat (A). Histogram in B compares the durations of flexor activity and flexor non-activity for situations where the extensor burst disappeared spontaneously ("No ext EMG") or was preset ("Control"). Average flexor burst duration (N = 18) = 692 msec (SD = 93) for "No ext EMG" and 498 msec (SD = 46) for "Control".

has to be fixed, which introduces some deformations of the normal locomotory output. Ideally one should apply graded electrical stimuli directly to the nerves of a limb which can move freely during locomotion. Such experiments have become possible since the development of electrode cuffs which can be implanted acutely or chronically around different nerves (Stein *et al.*, 1975).

### (1) *Acute experiments*

Only three of the four premammillary cats with implanted cuffs walked sufficiently well to produce consistent results and none walked well enough to allow much quantitative data treatment. Two cats had a cuff around the *posterior tibial nerve* (just above the ankle). The neural activity recorded from the intact nerve showed a very characteristic short burst of activity each time the pad touched the treadmill belt at the beginning of the stance phase (Fig. 20A). The sharp onset of this burst made it ideal as a marking point for the onset of the stance phase. Typically, activity in the ankle extensors started some 60 - 80 msec prior to the burst of neural activity at foot contact (Fig. 20A). This is in agreement with Engberg and Lundberg (1969), who showed that in the normal walking cat, extensor activity starts shortly before placing the foot on the ground.

The effects of short periods of tibial nerve stimulation on the locomotory output depended on the timing and intensity of the stimuli. Stimuli at 1.3 x T sometimes were sufficient to induce changes in the motor pattern but usually stimuli at 2 x T were needed.

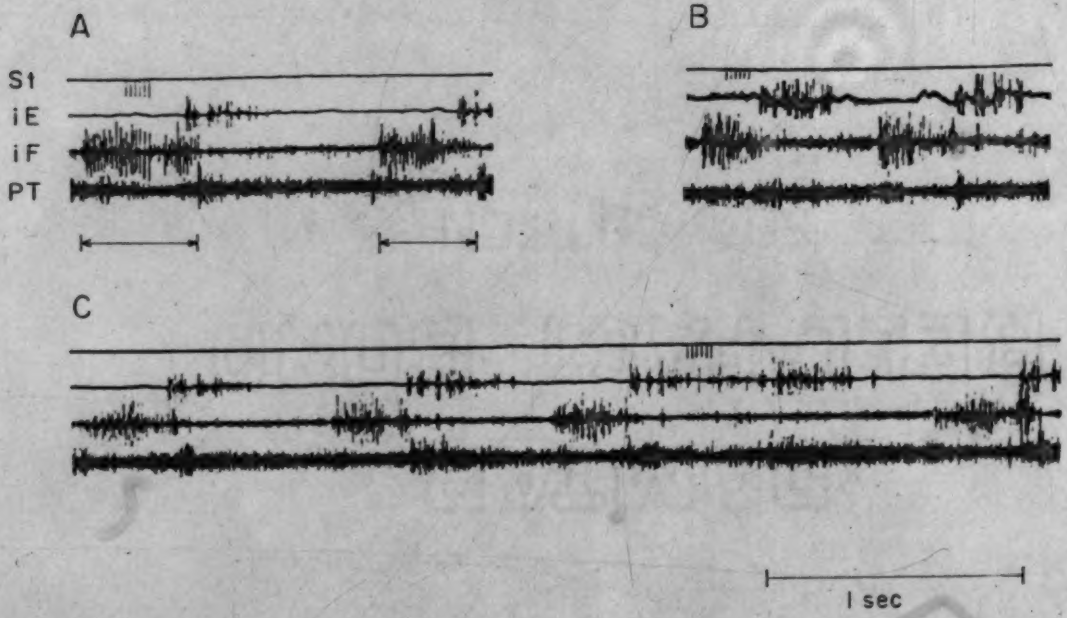


Fig. 20. Stimulation of the posterior tibial nerve in a walking premammillary cat. Stimulation during the flexion phase either prolonged (A) or shortened (B) the flexion phase, while stimuli applied during the extension phase yielded a prolongation of the extension phase (C). St = stimulus (six pulses of 0.05 msec at 60 Hz); iE = ipsilateral triceps surae; iF = ipsilateral pretibial flexors; PT = posterior tibial neural activity. Arrows: time between onset of flexor activity and neural PT burst corresponding to foot contact.

Fig. 20 illustrates the changes which could occur when the posterior tibial nerve was stimulated at 2 x T at different times of the step cycle.

Stimulation during the flexion phase (corresponding to the occurrence of a flexor burst) either prolonged or shortened the duration between the onset of flexion and the onset of extension (or onset of neural burst corresponding to foot contact). In the case of prolongation (Fig. 20A) the flexor activity was temporarily suppressed after a period of 40 - 50 msec following the onset of the stimulus train but flexor activity reappeared after the suppression. In the case of shortening (Fig. 20B) the flexor burst was not necessarily affected but extensor activity appeared sooner than expected on the basis of following or preceding step cycles. For ten subsequent trials of stimulation during the flexion phase (from the experiment of Fig. 20B), nine showed a shortening and one a prolongation of the duration between onset of flexion and onset of extension (average shortening = -15%; S.D. = 11). When given during the extension phase (corresponding to the burst of activity in the ankle extensors), the stimuli often produced no effect at all but occasionally a prolongation of the extension phase occurred (Fig. 20C). It is noteworthy that the 2 x T stimulation clearly inhibited periodic flexor spasms, occurring when the quality of locomotion deteriorated. At rest, the stimuli could evoke a flexor reflex.

In general, the changes in locomotory output were much less prominent than in the stimulation experiments with fixed hindlimb, presumably because in the latter experiments there was less competition of afferent input from different sources due to the immobilization. Nevertheless, the results obtained with cuff electrodes on the posterior tibial nerve seem to confirm, in general, the results obtained with hook electrodes (previous section). However, in the cuff experiments no clear breakpoint was found so far between evoked flexor burst prolongations and evoked flexor burst shortenings.

In one cat a cuff was implanted around the *common peroneal nerve*. Only strong shocks were applied, causing a flexor reflex during the periods of walking. This led to a drastic reduction in the duration of the contralateral flexor burst which was shortened to 48% of its normal value (average of 50 trials). Once the ipsilateral withdrawal reflex was terminated, the duration of the contralateral flexor burst returned to normal.

### (2) *Chronic experiments*

#### (a) *Posterior tibial nerve*

Two normal cats had a cuff installed around the posterior tibial nerve above the ankle but below the junction of the triceps surae nerves. When stimulated during locomotion both cats showed the same type of reaction but most data were collected on one cat, represented in Figs. 3 and 21.

Stimulation of the posterior tibial nerve at 1.4 x T *during the flexion phase* caused the onset of the activity in the ankle extensors to be delayed, especially when stimuli were applied at the end of the flexion phase (Fig. 21 left). This extension delaying effect was much more pronounced when the stimulus strength

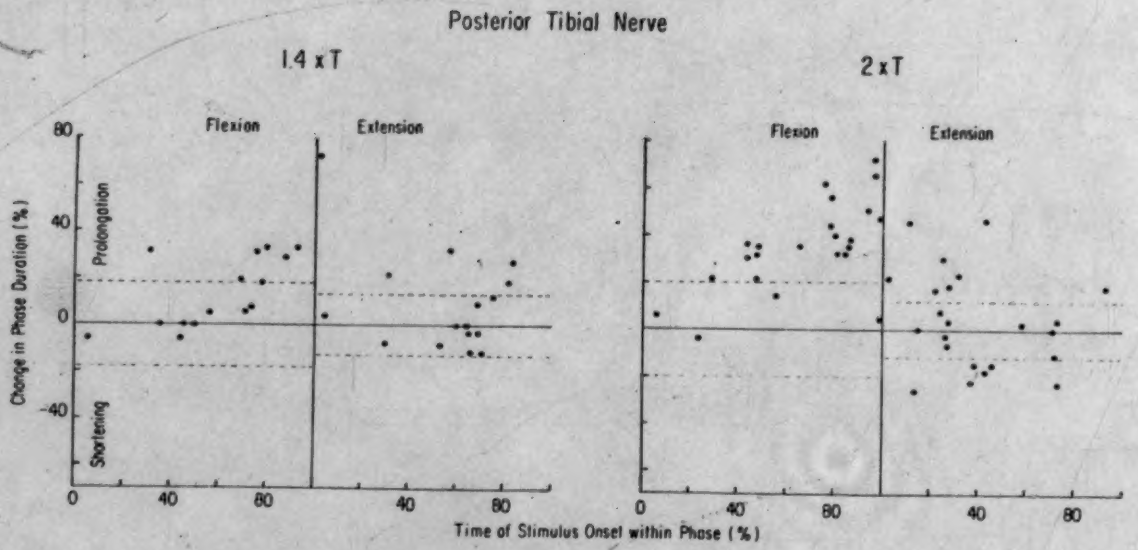


Fig. 21. Effect of posterior tibial nerve stimulation on the duration of the step cycle phase during which stimulation is applied. Stimuli: six pulses (0.05 msec) at 60 Hz. Pooled data from six experimental sessions (one preceding the cutting of the nerve and five during the month following the cutting).

was raised to 2 x T (Fig. 21, right). Since no flexor EMG was available the flexion phase was defined as the pause between two consecutive bursts of activity in the ankle extensors. To check if the prolonged flexion phase really corresponded to increased periods of flexion during the swing phase, high speed cinematography was used to verify the movements of the foot following stimulation. As shown in Fig. 3, the distance between the treadmill belt and the most distal point of the limb increased by about 100% following stimulation of the posterior tibial nerve at the end of the flexion phase. There is thus no doubt that the prolongation of the flexion phase corresponded with an additional flexion movement of the stimulated hindlimb.

The effects of stimulation of the posterior tibial nerve *during the extension phase* were very dependent on the stimulus strength. At 1.4 x T there was a tendency for the extension phase to be prolonged by the stimulation. At 2 x T there were two types of reactions following stimulation: either there was an immediate placing of the contralateral hindlimb followed by a flexion of the stimulated hindlimb (causing the ipsilateral extension phase to be shortened) or else the stimulated hindlimb "sagged" for a short time after which extension resumed (causing the total duration of the extension period to be prolonged; Fig. 3, bottom). The occurrence of the latter response was probably related to the contralateral hindlimb not being ready to take up the support of the animal but not enough filmed material was available to prove this point. On the other hand, the filmed data clearly showed that the stimulation can induce a drastic shortening of the contralateral flexion movement. The effects of posterior tibial nerve stimulation on the phase following the phase during which stimulation was started were very variable and statistically not significant (see methods).

(i) *Single pulses*

The application of trains of stimuli has the disadvantage that a long stimulus artefact is produced, making it often impossible to distinguish between EMG signals and stimulus artefact. This is the reason why Fig. 21 contains so few data points corresponding to stimulus application during the last 20% of the extension phase.

When single pulses are used instead of trains of pulses, one can reduce the duration of the stimulus artefact but higher stimulus intensities are required to obtain behavioral effects. As shown in Fig. 22, single pulses at 3 x T applied to the posterior tibial nerve had little effect on the duration of the flexion phase but markedly prolonged the duration of the extension phase, especially when applied during the latter parts of the extension phase.

(b) *Sural nerve*

Stimulation of the sural nerve in the *normal cat* with implanted cuffs around the sural, common peroneal and sciatic nerves yielded results as shown in Fig. 23. At rest, a train of pulses at 1.1 or 1.2 x T evoked a reflex activation in the extensor EMG picked up by the common peroneal cuff overlying the lateral gastrocnemius muscle (Fig. 23). During locomotion the same stimuli could cause a

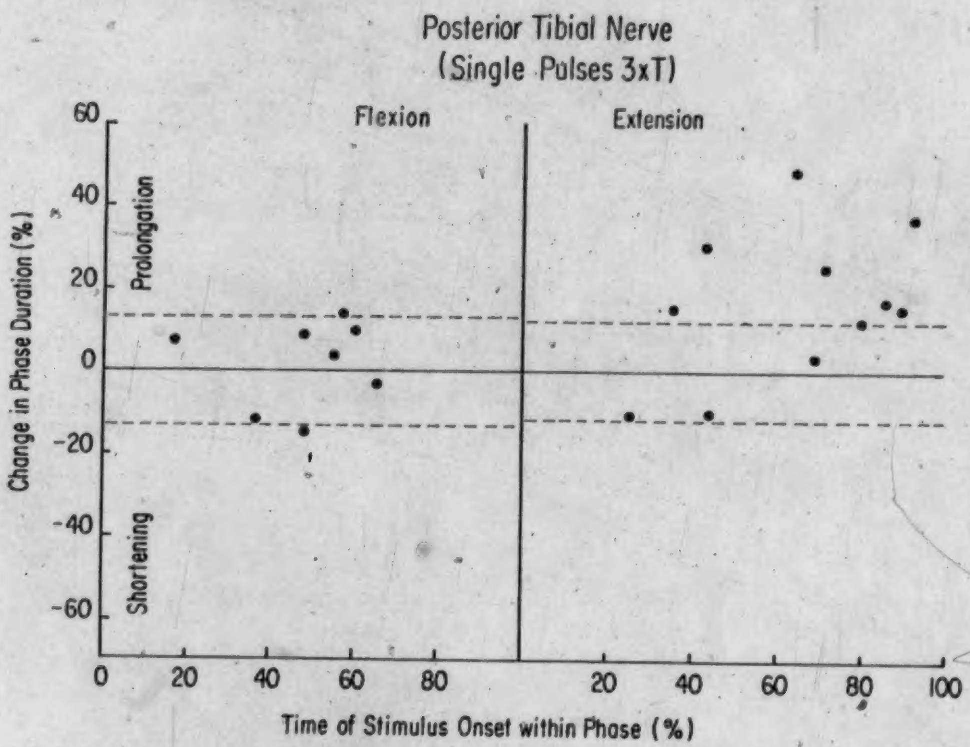


Fig. 22. Effects of single pulses to the posterior tibial nerve on the duration of the step cycle phase in which stimulation is applied. Construction of plot as in Fig. 21. Data based on one experimental session, 2 months after cutting the nerve. Note prolongation of extensor phase for stimuli applied after the first 40% of the extension phase.

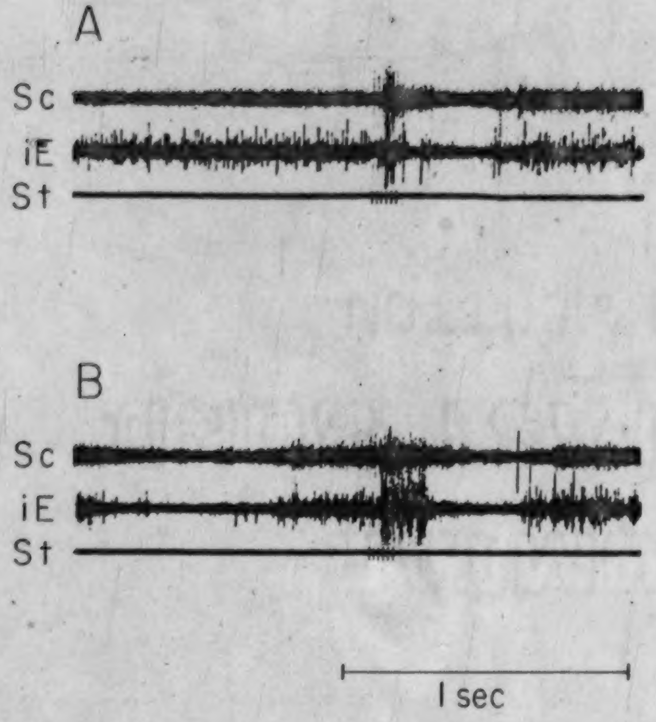


Fig. 23. Sural nerve stimulation (1.2 x T) evoking a triceps surae reflex in the normal resting cat (A) and in the walking cat during the stance phase (B). Sc = neural activity in the sciatic nerve; iE = ipsilateral triceps surae; St = six pulses of 0.01 msec at 60 Hz.

dramatic increase in the amplitude of the extensor EMG bursts at least when stimuli were applied during the extension phase (Fig. 23B).

If the changes in the duration of the flexion and extension phases were plotted versus the time of stimulus application, a plot as seen in Fig. 24 was obtained. Stimulation during the flexion phase tended to shorten the duration of the flexion phase, while stimulation during the extension phase generally shortened the extension phase when given at the beginning of the extension phase (probably correlated with the first extension phase) but prolonged the extension phase when given near the middle of the extension phase. Due to the stimulus artefact it was not possible to examine the effects of stimulus trains started in the last part of the extension phase.

### (c) *Common peroneal nerve*

In one normal cat the cut common peroneal nerve was stimulated with 1.1 x T at rest and during locomotion. The EMG of the ankle extensors, recorded from outside the cuff around the sural nerve, showed a reflex activation in the resting animal (Fig. 25, top). This reflex activation was much more conspicuous when the stimulation was applied during the stance phase (Fig. 25, middle). The increased amplitude of the EMG burst from the ankle extensors during the extension phase was sometimes correlated with a shortening of the extensor burst but sometimes a prolongation occurred.

In addition, stimulation of the cut common peroneal at 1.1 x T during the extension phase evoked at times a reaction similar to what Sherrington (1906) described as the "shake reflex" (Fig. 25,

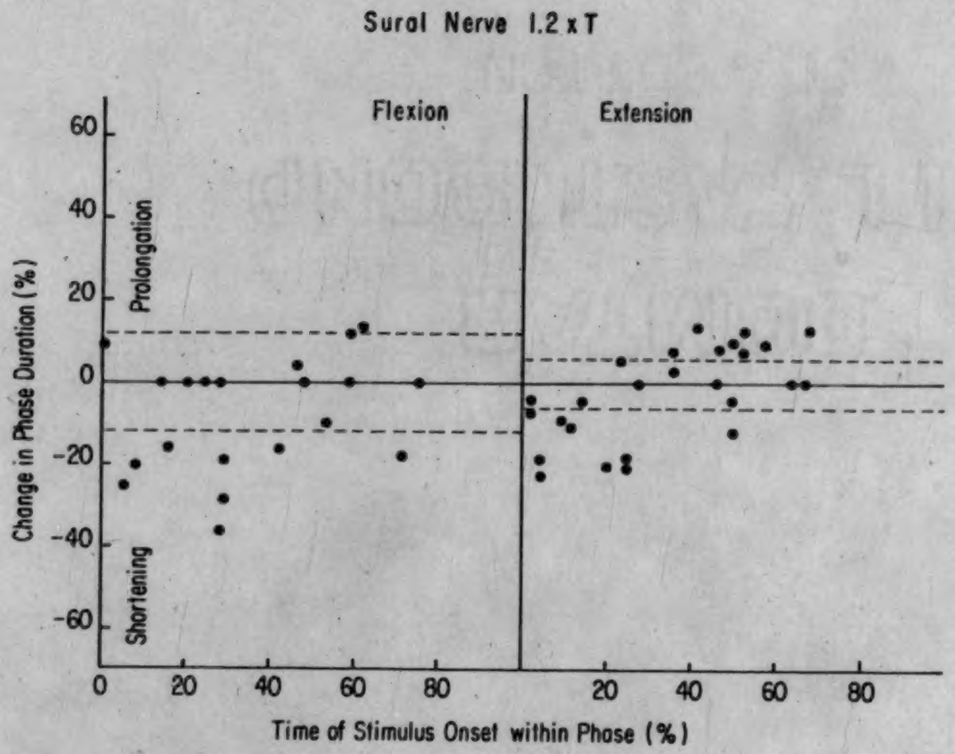


Fig. 24. Effect of sural nerve stimulation on the duration of the flexion and extension phases of normal walking cat. Stimuli: six pulses (0.01 msec) at 60 Hz. Large stimulus artefact prevented analysis of stimulus trials where pulse train was started at the end of flexion or extension phase. Data from two experimental sessions.

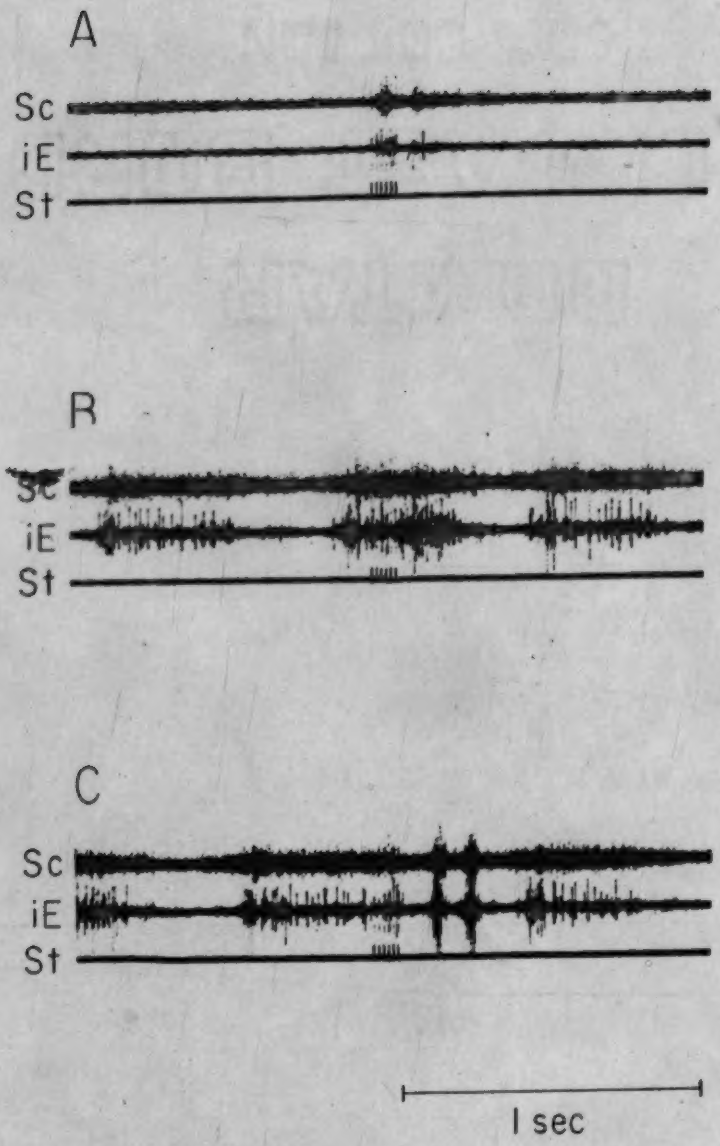


Fig. 25. Stimulation of the intact common peroneal nerve at 1.1 x T in the normal cat with implanted neural cuff. (A) at rest, (B) and (C) during the stance phase of walking. Same symbols and same cat as in Fig. 23.

bottom). Before initiating the swing phase the limb was shaken a few times as if to shake some imaginary water from its foot.

## 2.4 *Discussion*

### 2.4.1 *The extensor reflex and the extension phase*

Electrically stimulating the pad or the plantar surface of the foot of a resting premammillary cat evokes a reflex contraction of the ipsilateral ankle extensors, while the same stimuli applied during the hindlimb stance phase of a walking cat prolong the ongoing stance by extending the period of extensor activity and delaying the following flexion. These observations support the idea that extensor reflexes elicited from the skin of the distal limb indeed participate in locomotion (see introduction). The actions from the pad and plantar side of the foot during the stance are essentially twofold: firstly, there is an increase in the intensity of the ongoing extension; secondly, there is a delay in the onset of the flexor activity. Both actions seem particularly useful for an animal walking over an unpredictable surface. An unexpected extra loading of the hindlimb during the stance phase will provide extra stimulation of the cutaneous receptors of the pad or the plantar surface of the foot, and this in turn will lead to a reflexly evoked increase in extensor activity, counteracting the load. Furthermore, the presence of extra stimulation to the pad or the plantar surface of the foot will delay the moment at which the foot is lifted from the ground (swing).

The results obtained with stimulation of the pad and plantar surface of the foot could be duplicated in premammillary cats using electrical stimulation of the nerves innervating these skin areas (Table 1). In addition, the nerve stimulating experiments indicated that the extensor reflexes in the resting and walking animal are critically related to the activation of the larger afferents within these nerves. Strong connections from low threshold sural nerve afferents to triceps alpha (Hagbarth, 1952; Wilson, 1963; Burke *et al.*, 1973) and gamma motoneurons (Eldred and Hagbarth, 1954; Hunt and Paintal, 1958; Barrios *et al.*, 1969) are known to exist. Moreover, monosynaptic ankle extensor reflexes are facilitated by volleys in the sural nerve (Bernhard, 1947). However, the latter facilitation of triceps surae reflexes is not essential in the explanation of the present results since cutting the nerve to the triceps surae did not prevent the prolongation of the step cycle and the delay of the next flexor burst when the sural nerve was stimulated with 2.5 x T (hook electrodes).

In the normal cat with implanted cuff electrodes it was found that low threshold stimulation of the sural (1.2 x T) and posterior tibial nerve (1.4 x T) increased the amplitude and/or duration of the EMG activity in the ankle extensors during the stance. In addition, however, it was noted that the same effect could be obtained with low threshold stimulation of the common peroneal nerve (1.1 x T), which indicates that even large afferents from the dorsum of the foot may provide additional extensor excitation during the stance. This is consistent with the results of Forssberg *et al.* (1975), who found that stimulation of the dorsum of the foot during

| | Time of onset of stimulus within step cycle | | | | | |
|---|---|---|---|---|---|---|
| | Early F Phase | | Late F Phase | | E Phase | |
| 1. Pad and Plantar Surface | F burst | ↑ | F burst | ↑ | Next F burst | = |
| | Next E burst | ↓ | Next E burst | ↓ | E burst | ↑ |
| | Cycle | =↑ | Cycle | ↓ | Cycle | ↑ |
| 2. Hook Electrodes | F burst | ↑ | F burst | ↓ | Next F burst | = |
| Tibial/Sural Nerve | Next E burst | ↓ | Next E burst | ↑ | E burst | ↑ |
| A. Weak Stimuli | Cycle | =↑ | Cycle | ↓ | Cycle | ↑ |
| B. Strong Stimuli | F burst | ↑ | F burst | ↑ | Next F burst | ↑ |
| | Next E burst | ↓ | Next E burst | ↓ | E burst | ↓ |
| | Cycle | =↑ | Cycle | ↓ | Cycle | ↓ |
| 3. Cuffs (Chronic) | | | | | | |
| - Post. Tibial N (2xT) | F phase | ↑ | F phase | ↑ | E burst | ↑ |
| - Sural N (1.2xT) | F phase | ↓ | F phase | ↓ | E burst | ↑ |
| - Common Per. (1.1xT) | | | | | E burst | ↑ |

TABLE 1

Changes in duration of step cycle phases produced by stimulation of cutaneous afferents the stance phase increased the amplitude of the extension in chronic spinal cats.

The fact that extension promoting effects were obtained from many different nerves in the adult cat is probably a remnant of the strong and widespread cutaneous extensor reflexes of the immature kitten. According to Ekholm (1967), the source of the most effective excitation of extensor motoneurons shifts postnatally from cutaneous exteroceptors to muscle proprioceptors, a change which coincides with the development of the gamma motor system. It might then be expected that cutaneous reflexes have evolved to occupy functions in parallel with proprioceptive reflexes, appearing at a later stage. In the adult animal extensor reflexes are indeed readily evoked by stimulating muscle nerves as well as cutaneous nerves (Sherrington and Sowton, 1911). A close parallelism in the actions from exteroceptors and proprioceptors is likely to explain why so little deficit was seen in the experiments with denervation or anesthesia of the skin of the foot (Sherrington, 1910; Engberg, 1964).

### 2.4.2 *Reflex modulation of the flexion phase*

When the same stimuli which evoked an increase in the amplitude and/or duration of the extensor activity during the stance were applied during the swing phase, two types of results were obtained (Table 1): either the flexion phase was shortened or else it was prolonged.

### (A) *Shortening of the flexion phase*

In the acute premammillary cat with fixed hindlimb, low intensity stimulation of the tibial or sural nerve at the end of the flexion phase was seen to reduce the duration of the ongoing burst of activity in the pretibial flexors while at the same time inducing a premature onset of the EMG burst in ankle and hip extensors (triceps surae and semimembranosus; Fig. 13). This observation could hardly have been predicted on the basis of reflex pathways unveiled by experiments on immobile animals. Hamstring muscles in anesthetized cats receive reflex excitation by low intensity stimulation of the nerves to ankle and toe extensors (Eccles *et al.*, 1957) but are not known to receive excitatory connections from large sural nerve afferents. It thus seems that there may be fundamental differences between the central reflex actions of walking and immobile animals. Presumably this is due to reflex connections with spinal coordinating centers which are activated during locomotion but inactive in the immobile animal. Centers of this type have been termed "half-center" by Graham Brown (1914). Originally the term "half-center" was used sometimes to designate motoneuronal pools, but when the term was reintroduced by Jankowska *et al.* (1967b) it was used in the sense of mutually inhibitory interneuronal networks intercalated between primary afferents and motoneurons. In keeping with Jankowska's use of the term, "Flexor Half-Center" (FHC) and "Extensor Half-Center" (EHC) will be used here to designate two mutually inhibitory groups of interneurons responsible for the generation of rhythmic bursts of activity in flexor and extensor muscles respectively. So far then, the present results can be explained if large cutaneous afferents in the sural, tibial and peroneal nerves make inhibitory connections with the FHC and excitatory connections with the EHC, since such connections can account for the prolongation of the extension phase when stimuli are given during the stance and for the shortening of the flexion phase when stimuli are given at the end of flexion.

The question remains why the flexor burst shortening for stimuli given during the flexion phase was so much more obvious in the experiments with fixed hindlimb. As already pointed out in the "results" section, the answer is related to the fact that fixing the hindlimb increased the relative duration of the flexor burst within the step cycle. Secondly, the situation with fixed hindlimb is a simplified situation in that there is a drastic reduction in the total amount of afferent input due to the fixation. Nevertheless, it seems that large cutaneous afferents may also inhibit the FHC in animals with freely moving limbs since a shortening of the flexion phase was found in normal chronic cats with a cuff implanted around the sural nerve. On the other hand, the chronic experiments with a cuff around the posterior tibial nerve never yielded a shortening of the flexion phase for stimuli applied during flexion (Table 1). It has to be kept in mind, however, that some large afferents may have been damaged preferentially due to mechanical block in the chronically implanted cuffs (Stein *et al.*, 1976). Moreover, the cutting of these nerves in the latter experiments may also have contributed to a loss of larger afferents since there was a decrease in the conduction velocity after denervation (Stein, personal communication). One must be cautious, therefore, in comparing the thresholds of the nerves in the normal and acute animal, and it is quite possible that the 1.4 x T and 2 x T stimulation in the normal cat activated a lot more small afferents than stimulation with the same parameters in the acute cat.

What then is the functional significance of the shortening of the flexion phase by activation of large cutaneous afferents? If the foot touches the ground earlier than expected during the swing phase, then it is of importance for the animal to initiate immediate extension so that the limb can take up the support of the animal as fast as possible. Perhaps this also explains why large cutaneous afferents mainly excite the large fast triceps surae motoneurons instead of the slow ones (Burke *et al.*, 1970).

### (B) *Prolongation of the flexion phase*

Instead of shortening the flexion phase after unexpectedly touching an object, the animal may instead exaggerate its flexion so as to overcome the object (Forssberg *et al.*, 1975). The result could be a prolongation of the flexion phase, and this was indeed observed on many occasions in the present experiments (Table 1). Of special interest were the observations on premammillary cats with fixed hindlimb since there the flexor burst prolongations were limited to weak stimuli applied at the beginning of the flexion phase while the same stimuli applied later in the flexion phase produced a flexor burst shortening. Moreover, it was found in the latter experiments and in the experiments with pad stimulation as well that a flexor burst rebound could be evoked instead of a flexor burst prolongation. The two phenomena may thus be related and since the presence of rebound is indicative of inhibition, one should not use the finding of flexor burst prolongation as evidence that only excitatory actions are exerted onto the structure responsible for the generation of flexor bursts (FHC). Indeed, inhibitory influences may not always be apparent and rebound may occur even without any sign of inhibition (Brown, 1911a; Sherrington, 1913). The results on flexor burst prolongation may thus be compatible with the previously mentioned hypothesis that large cutaneous afferents make inhibitory connections with the FHC and excitatory connections with the EHC.

It is of interest that the present results on hyperflexion due to stimulation of the tibial nerve at the end of the flexion phase in normal cats agree particularly well with the observations made by Forssberg *et al.* (1976) on chronic spinal cats. In the latter study it was found that electrical stimulation of the dorsum of the foot during the swing phase results in a brisk flexion of the knee and ankle (and to some extent the hip). Hence, both in the spinal and normal cat the motor program of the flexion phase can be altered by an additional evoked flexion movement of the *whole limb*. In the spinal cat, this extra flexion movement does not necessarily change the total duration of the swing phase.

In summary, prolongations of the flexion phase may occur either with cutaneous stimulation at the very beginning (Figs. 5 and 13) or at the very end of the flexion phase (Fig. 21). The prolongations *at the beginning* of the flexion phase were obtained mainly with weak cutaneous stimuli and can be understood functionally as an "overstepping" reaction of an animal touching an object in the early course of the swing phase. Termination of the swing phase at an early stage is probably very difficult, if not impossible, in view of the rigid central programming of the early period of the swing phase (Orlovsky and Shik, 1965).

On the other hand, the prolongation of the flexion phase for stimuli applied *at the end* of flexion (Fig. 21) is likely to be more related to the withdrawal reflex. The touching of harmless unexpected elevations of the ground may lead to a shortening of the flexion phase (see discussion above), but if a pain eliciting object is touched then the best reaction for the animal is to delay the placing of the foot. The idea that these late prolongations of the flexion phase are related to a withdrawal reflex was substantiated in the normal cat by the fact that the same stimuli applied during the stance also produced a flexion reflex leading to the "sagging" of the animal.

### 2.4.3 *The flexor reflex and locomotion*

In the acute experiments with fixed hindlimb it was found that the extensor burst prolonging and the flexor burst shortening effects due to low intensity stimulation of the tibial or sural nerve were gradually replaced by extensor burst shortening and flexor burst prolonging effects when the stimulus intensity was increased. High intensity stimulation of these nerves prolonged the duration of the flexor burst if applied before or during the flexion phase, while stimulation before or during the extension phase yielded extensor burst shortenings (Table 1). Afferents having these effects on flexor and extensor bursts were actually found in all nerves examined and their presence was especially prominent in the common peroneal nerve. It is therefore likely that these fibers belong to the so-called "FRA" group. Since activation of these afferents leads to contractions in all flexors and relaxations in all extensors, it is likely that these FRA afferents make excitatory connections with the FHC and inhibitory connections with the EHC.

So far, only the actions of the FRA on individual step cycles have been discussed without mentioning the effects on the overall locomotory rhythm. The effects on the rhythm have been examined by applying strong stimuli (75 x T) to the sural nerve during locomotion. It was found that such stimuli caused a bilateral resetting of the stepping rhythm except if given just prior to the onset of the ipsilateral flexion phase. The absence of resetting effects at the end of the extension phase may be explained in two ways. First, the rhythm generating system may be closed to FRA input at the end of extension, and, secondly, the rhythm generating system may be open to FRA input at all times but its functioning may be disrupted only if FRA input arrives at an inappropriate time of the cycle. The observation that stimuli applied at the end of the extension phase may still prolong the following flexor burst by at least 20% argues against the hypothesis that FRA activity is

6 7

somehow "gated" centrally. It should be noted that the step cycle contains a second period during which stimulation has minimal resetting effect. Since this second period precedes the onset of ipsilateral extension and contralateral flexion, it is possible that in this case the minimal resetting is the result of excitatory connections with the ipsilateral EHC and the contralateral FHC.

### 2.4.4 *Model of central connections of large cutaneous afferents* (Fig. 26)

From the previous discussion it is evident that large cutaneous afferents from the sural and tibial nerve have central connections which must differ from those of small cutaneous afferents. Nevertheless, both large and small cutaneous afferents are still classified as FRA because of observations made on the immobile spinal cat (Holmqvist and Lundberg, 1961) in which the pathway from large cutaneous afferents to flexor motoneurons is open. However, this path is inhibited in walking animals where the excitatory path to extensors and the inhibitory path to flexors are much more important. It thus seems very misleading to classify the large cutaneous afferents and the small afferents as a single functional unit.

Let us now consider the "alternative" pathways from large cutaneous afferents to flexor and extensor motoneurons in relation to locomotion (Holmqvist and Lundberg, 1961). One such pathway, called "fast" pathway, excites flexor motoneurons while at the same time inhibiting monosynaptic reflexes to extensor motoneurons. This

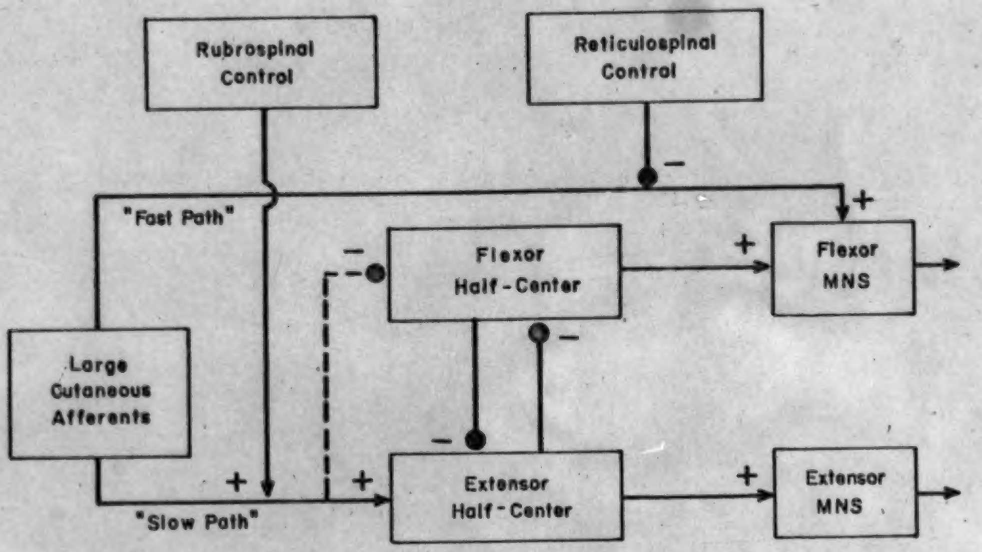

Rubrospinal Control
Reticulospinal Control
"Fast Path"
Flexor Half-Center
Flexor MNS
Large Cutaneous Afferents
Extensor Half-Center
Extensor MNS
"Slow Path"
+
−

Fig. 26. Diagram illustrating some central connections made by large cutaneous afferents in the tibial and sural nerves. Further explanation in text.

fast pathway is characteristically inhibited by maneouvers which induce locomotion, such as intravenous injection of DOPA (Andén, 1966; Jankowska *et al.*, 1967a) or Clonidine (Grillner, 1973b) in the spinal cat or stimulation of the MLR in the mesencephalic cat (Grillner and Shik, 1973). Sural nerve stimulation under these conditions gives rise to long latency and long lasting flexor discharges which typically appear after termination of the stimulus train, irrespective of the duration of this train. Since the latter behavior is very typical for rebound as it was also observed in the present experiments, it seems reasonable to assume that these late flexor discharges arise in a structure similar or identical to the FHC.

If one accepts this assumption, then the present data and the data on late flexor discharges can be explained on the basis of a diagram such as depicted in Fig. 26. A fast pathway to flexor motoneurons is inhibited by a central command related to locomotory behavior (DOPA, Clonidine, brainstem stimulation, spontaneous). As a result, activity in large cutaneous afferents now follows mainly the excitatory pathway to the EHC, which strongly inhibits the FHC. Abrupt termination of the FHC inhibition induces rebound in the FHC, resulting in long late flexor discharges or flexor burst rebound. According to this hypothesis, late flexor discharges would be expected to be preceded by a period of extensor activity, as was indeed observed by Jankowska *et al.* (1967a). However, it is possible that the FHC inhibition is direct rather than through the EHC and this particular aspect of the diagram awaits confirmation from experiments showing that the EHC excitation precedes the FHC inhibition. Whether or not the FHC inhibition is direct or indirect, one can at least exclude the possibility that the slow path by-passes the FHC or EHC since such a by-pass to extensor motoneurons would fail to explain the observation that stimulation of large cutaneous afferents during the extension phase not only prolongs the extension phase but also delays the onset of the following flexor burst, which is unchanged in duration.

The different pathways from the large cutaneous afferents are under supraspinal control. Stimulation of the red nucleus was shown to excite predominantly fast twitch units in the triceps surae, presumably through activation of the same interneurons that transmit excitation from low threshold cutaneous afferents to these motor units (Baldissera *et al.*, 1971; Burke *et al.*, 1970). On the other hand, stimulation of the dorsal reticulospinal system is known to be very effective in suppressing short-latency FRA pathways (Engberg *et al.*, 1968). Since neurons in both the rubrospinal and reticulospinal system reach their peak activity at the transition from flexion to extension during the swing phase (Orlovsky, 1970, 1972; Wetzel and Stuart, 1976), it seems likely that activity in these supraspinal neurons may have contributed to the flexor-extensor transition effects observed in our experiments with low intensity sural and tibial nerve stimulation.

One of the implications of Fig. 26 is that the resting animal may have both pathways in use at the same time. Such interpretation would explain the finding of an early and a late component of the flexor reflex as well as the observation that an extensor reflex lies concealed under the flexor reflex. Functionally, a model such as shown in Fig. 25 is very meaningful. Fast flexor reflexes evoked from large cutaneous afferents may be useful for the protection of the resting animal, but they seem inappropriate in the walking animal where they would severely disrupt the ongoing motor activity. On the other hand, it would seem of crucial importance for the walking animal to be able to terminate flexion and initiate extension as fast as possible if foot contact is made at the end of the flexion phase in walking.

### 2.4.5 *The central programming of the flexor burst; similarities with the respiratory system*

The demonstration in premammillary cats that the initial part of the flexor burst is much more resistant to inhibitory input indicates that this initial part is quite strictly centrally programmed. A similar situation is found in the respiratory system where the initial part of the inspiratory burst of activity is more resistant to inhibitory input from various sources. Electrical shocks applied to the vagus nerve (Boyd and Maaske, 1939), the superior laryngeal nerve (Larrabee and Hodes, 1948), the medulla (Burns and Salmoiraghi, 1960) or to part of the nucleus parabrachialis (Cohen, 1971) could either bring the activity of diaphragmatic units to an abrupt end or else cause a prolongation or rebound of this activity. In parallel with the present findings, these effects were dependent on timing and intensity of the applied shocks. Stronger shocks were needed to stop inspiration when applied at earlier times within the inspiratory phase, but a complete inhibition was never seen.

In this context, it is noteworthy that the locomotory flexor burst was similar not only to the inspiratory burst of activity in the phrenic nerve but also to the after-discharge of the flexor reflex which is easier to inhibit at later parts of its time course (Liddell and Sherrington, 1925). Further similarities between the flexor burst and the inspiratory burst concern their common shape (gradual build-up) and their standard duration under normal circumstances (Cohen, 1975; Orlovsky and Shik, 1965).

# CHAPTER 3. THE ROLE OF MUSCLE AFFERENTS IN LOCOMOTION

## 3.1 *Introduction*

It is generally accepted that feedback from muscle afferents must play an important role in walking, although there is some disagreement about the usefulness of proprioceptive reflexes at higher speeds of locomotion (Grillner, 1973a; Nichols and Houk, 1976). The main source of muscle afferent activity during walking of mesencephalic cats seems to arise from the muscles while they are contracting (α-γ coactivation; Severin *et al.*, 1967). The way this afferent activity is used by the nervous system remains largely an open question, although Lundberg (1969) has made some speculations based on his findings on reflex connections made by muscle afferents in immobilized cats.

In this chapter, experiments will be described in which the function of receptors of the extensor muscles will be investigated during locomotion. In particular, the question will be asked whether extensor muscle afferents fulfill a similar function as described for the large cutaneous afferents from the distal hindlimb, namely the control of the initiation of the flexion phase. Extensor muscle afferents share with pad afferents the ability to signal to the central nervous system whether a limb is sufficiently unloaded at the end of the extension phase to be flexed without endangering the stability of the walking animal. Consequently, one would expect that the onset of the flexion phase would be prevented or delayed if the extensor muscles were additionally loaded during the extension phase.

To achieve this experimentally, receptors within the triceps surae were activated by stretching the muscle or by inducing a contraction (ventral root stimulation or triceps surae vibration). In other cases the nerves to the triceps surae were stimulated tetanically. The effects of these manoeuvers on the timing and occurrence of the bursts of activity in the pretibial flexors were examined.

### 3.2 *Methods*

Experiments were done on premammillary cats, 26 of which showed periods of spontaneous walking. The preparation of these animals has been described in the previous chapter. Again, the left hindlimb was partially denervated to allow the fixing of the hindlimb to a frame on the treadmill.

To achieve natural stimulation of muscle receptors, the triceps surae of the fixed hindlimb was freed as much as possible and its tendon was attached to a force transducer by means of a thread through a cut part of the calcaneus bone. The force transducer was connected in series either with a manually controlled lever system (10 cats) or a commercial vibrator which allowed for constant periods of vibration with adjustable amplitude (5 cats). In other experiments (N=5) a laminectomy was performed which allowed for electrical stimulation of ventral root filaments or for recording from dorsal root filaments. In 3 cats the four hindlimbs were left free but an adjustable metal clamp was attached to the left ankle with a screw through the tibia and a pressure clamp around the deafferented foot. A bolt in a semicircular slit allowed the ankle to be fixed at any desired angle.

Nerve stimulation was done in the premammillary cat with fixed hindlimb (N=8) or in the normal cat with implanted cuff (N=1) using the same techniques as described in the previous chapter.

## 3.3 *Results*

### 3.3.1 *Dorsal root recording*

While recording from single dorsal root filaments in walking mesencephalic cats, Severin *et al.* (1967) found that spindle afferents in extensor muscles fire mainly during the extension phase.

In a few premammillary cats we were able to substantiate their conclusions. Fig. 27 shows activity in a single filament from dorsal root L7. Activity in this filament increased when the ankle was flexed passively. When the triceps surae was made to contract by stimulating the muscle through two implanted wire electrodes, the afferent activity stopped temporarily for 40 - 50 msec (Fig. 27A). On this basis the afferent was identified as a triceps surae spindle afferent. During locomotion the activity in this afferent fiber was clearly correlated with the periods of activity of the triceps surae (Fig. 27B). In agreement with the results of Severin *et al.* (1967), the onset of the spindle afferent activity followed the onset of the EMG activity and persisted usually for a short period after the cessation of the extensor EMG burst.

It thus seems that in locomotion the principle of alpha-gamma coactivation holds for the premammillary cat as well as for the mesencephalic cat.

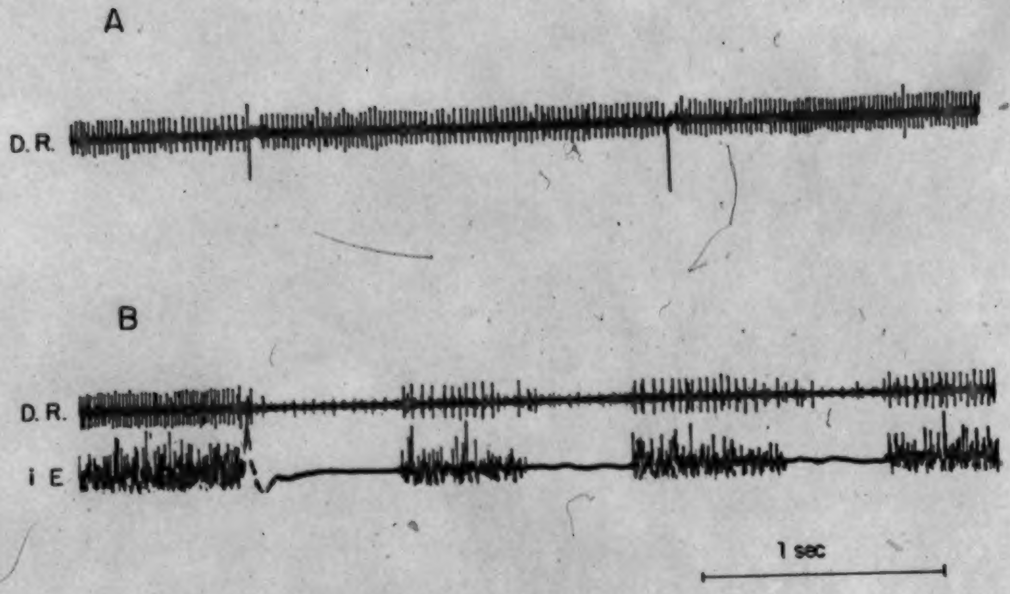


Fig. 27. Activity in ankle extensor spindle afferent at rest and during walking. (A) Identification of single unit in dorsal root (D.R.) filament (L7) by eliciting a contraction in the ankle extensors (iE); (B) Activity in the same unit at the transition from standing to walking. Premammillary cat.

### 3.3.2 *Natural stimulation*

#### (A) *All-or-none disappearance of flexor burst*

#### (1) *Triceps surae stretch*

The proposal that proprioceptive input from extensor muscles controls the functioning of the locomotor "oscillator" of a single limb arose from an observation made early in the course of the present experiments. It was noticed that restraint of one hindlimb of a walking thalamic cat or holding the ankle in a flexed position abolished the stepping movements of that hindlimb provided these manoeuvers were carried out during the stance phase (Fig. 28). This finding can be explained by assuming that the resistance to extension prevents the shortening of the extensor muscles, thus eliciting from these muscles a large amount of afferent activity which inhibits the "oscillator" of the restrained hindlimb.

To test the latter hypothesis it was necessary to design experiments in which it would be possible to evaluate selectively the effects of stretch of extensor muscles on the locomotor output. Two types of experiments were done. In the first type of experiment a gradually increasing stretch was applied to the triceps surae while in the second type of experiment a constant stretch of the triceps surae was used.

#### (a) *Graded stretch*

As shown in Fig. 29, the increased stretch of the triceps surae in the left fixed hindlimb resulted in a gradual increase in

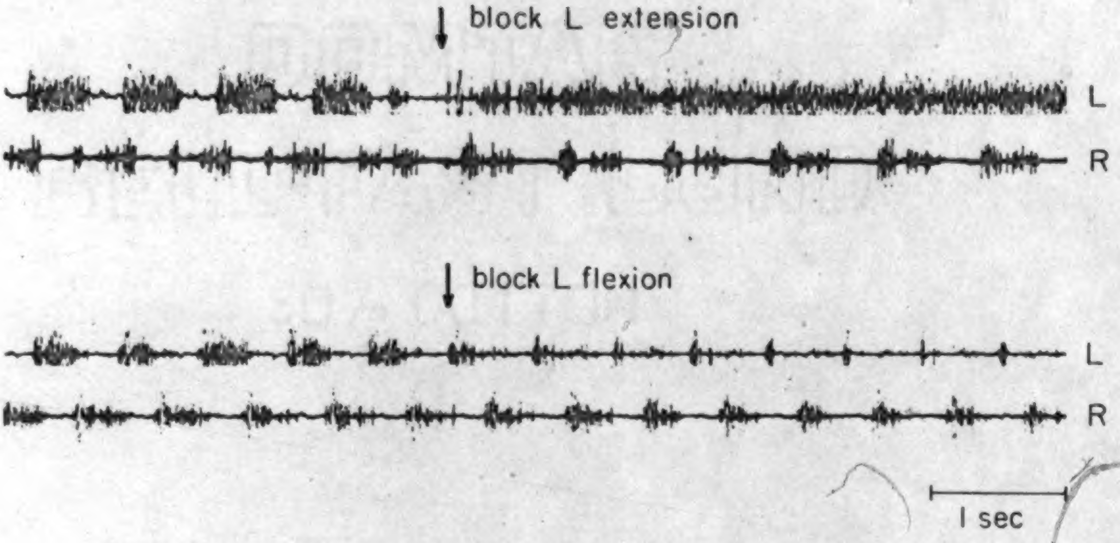


Fig. 28. Blocking of locomotory rhythm by stopping the hindlimb in extension. Preventing full extension inhibits the rhythmicity in the ipsilateral left hindlimb (L), while stepping continues at the right side (R). In contrast, preventing the hindlimb to fully flex does not abolished the ipsilateral rhythm.

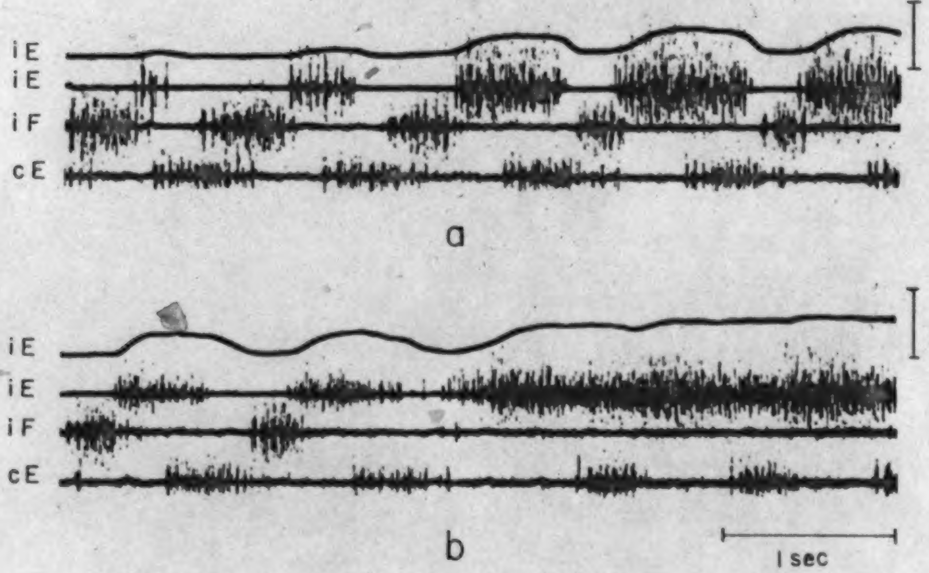


Fig. 29. Inhibition of rhythmic motor activity by stretch of the triceps surae of a walking premammillary cat. The increased stretch reduced the duration of the rhythmic flexor burst (a) until the burst disappeared altogether in an all-or-none fashion (b). iE = ipsilateral triceps surae force (top) and EMG (second trace); iF = ipsilateral pretibial flexors; cE = contralateral triceps surae. Note that there is a pause in the extensor activity despite the disappearance of the flexor burst (b). "b" from the same cat as "a" but filmed with lower gain of the iE EMG channel. Force calibration = 6.5 kg.

in amplitude and duration of the rhythmic bursts of activity in the stretched muscles until a tonic contraction replaced the rhythmic bursts. In contrast, the duration of the EMG bursts in the pretibial flexors was gradually reduced until a critical duration was reached and the flexor burst disappeared altogether. The flexor burst disappearance always occurred in a discontinuous "all-or-none" fashion, although the critical burst duration at which the disappearance occurred varied from experiment to experiment, as did the force required to block the rhythm. Most frequently the rhythmicity stopped when the increased force in the stretched muscles reached a level of 1.5 to 4 kg. These forces are within the physiological range for the triceps surae, according to Grillner (1973). However, in cats showing very powerful walking, the force required to stop the rhythm could go as high as 8 kg. On the contralateral side stepping continued despite the blocking of the ipsilateral rhythm.

### (b) *Isometric stretch*

A possible objection to the previous data is that the stretch of the triceps surae may have caused a blocking of the locomotory rhythm only in the muscles at the ankle. Also, the abnormal restraint of the hindlimb may have had some influence on the observed effects. To answer this criticism, 3 cats had a special device attached to the left ankle, allowing the ankle to be clamped at different angles without interfering with the movements at the other joints (see methods). With the clamp loose, movements at the ankle were normal (Fig. 30A), but fixing the ankle at an angle less than 90° resulted in prominent changes in the structure of the step cycle. At an angle of 55° the ipsilateral triceps surae contractions took up a much larger percentage of the step cycle than before. In contrast, the bursts of activity in the pretibial flexors were much shorter or occasionally disappeared altogether in an all-or-none fashion. Again, the disappearance of the flexor burst could occur without the elimination of an EMG silence in the extensors. Clamping the ankle angle at even smaller angles (40°, Fig. 30B) completely abolished all rhythmic activity not only at the fixed ankle but also at the other joints of the same limb. The whole left hindlimb was hyperextended (hip angles close to 150°), and a strong tonic contraction developed in all extensors. The three other limbs continued stepping. The thresholds for these effects showed considerable inter- and intra-animal variability. Thus, for the 3 cats a complete block of the ipsilateral hindlimb rhythmicity was obtained at angles of 45°, 75° and 70° respectively. During normal locomotion such ankle angles are rarely used except in situations where the animal is forced to crouch (Goslow *et al.*, 1973). Nevertheless, these experiments demonstrate that (a) the rhythmicity in a single limb can be blocked without stopping the walking of the three other limbs, and (b) an all-or-none disappearance of the rhythmic flexor burst can be elicited by tonic activation of afferents from the triceps surae (the foot being deafferented).

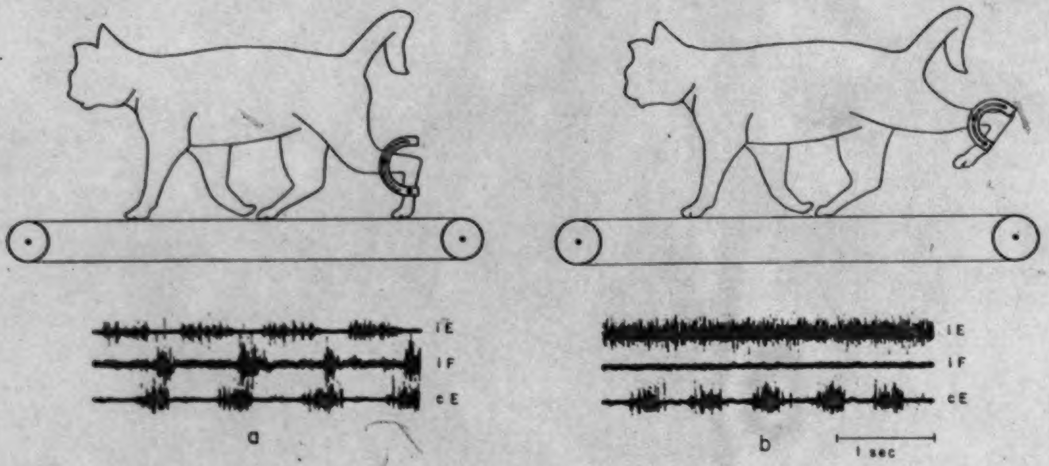


Fig. 30. The inhibition of ipsilateral hindlimb rhythmicity due to clamping of the left ankle in a flexed position. (a) = clamp loose; (b) = clamp fixed at 70°. Observe the hyperextension of the non-stepping limb with the clamped ankle (b top). Foot in clamp was denervated. iE, iF and cE as in previous figure.

### B. *Triceps surae contractions evoked by ventral root stimulation or triceps surae vibration*

Muscle receptors can be activated in a natural way not only by muscle stretch but also by evoking contractions in the muscles containing these receptors. For this purpose, a laminectomy was performed in 3 partially denervated cats and the distal part of a section of ventral root L7 was periodically stimulated with a train of supramaximal stimuli. As shown in Fig. 31A, the stimuli given during the extension phase caused a contraction of the ipsilateral triceps surae and this contraction was superimposed on the ongoing contraction, leading to the complete suppression of the burst of activity in the pretibial flexors. The timing of the stimulus application was of major importance to elicit this suppression effect since stimulation started earlier in the flexor burst was ineffective. The suppression happened in an all-or-none fashion and there was no gradual transition from a complete to a partial suppression of the flexor burst.

Another physiological way to obtain a muscle contraction is by eliciting a TVR (Tonic Vibratory Reflex). In 5 cats, brief periods of vibration (100 - 400 msec) at 64 Hz were applied to the isometrically held left triceps surae which, as a result, contracted reflexly. The periods of vibration were then repeated when the animals were walking. As seen in Fig. 31B, vibration applied in the extension phase could lead to the all-or-none suppression of the flexor burst in much the same way as was previously seen for the ventral root stimulation.

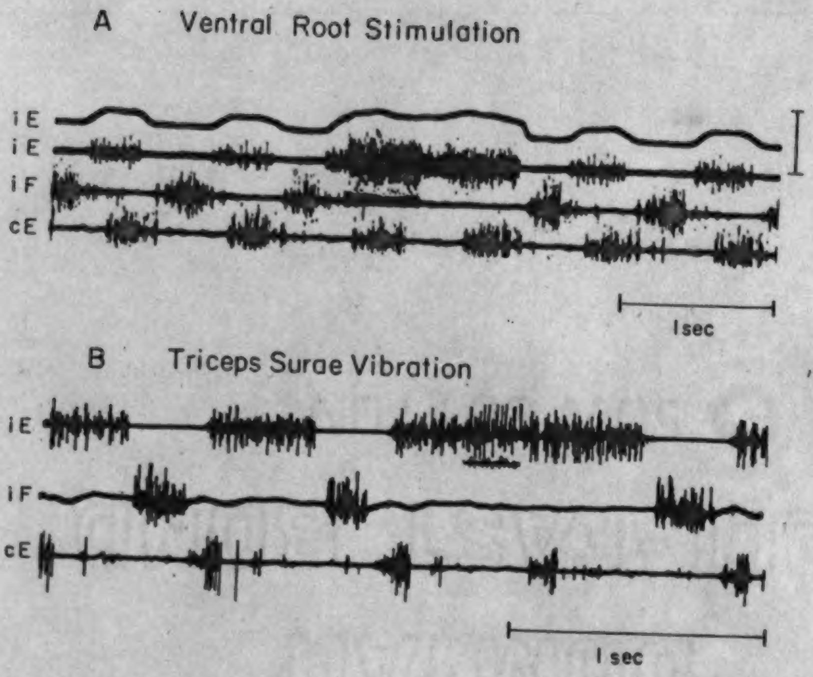


Fig. 31. All-or-none disappearance of the rhythmic flexor burst due to stimulation of an L7 ventral root filament (A) or to vibration of the triceps surae (B). iE, iF and cE as in Fig. 29. Stimuli (A): 480 msec train of pulses at 90 Hz, supramaximal for iE contraction; note large stimulus artefact in iE and iF. Vibration (B): 200 msec period at 64 Hz indicated by rippled bar taken from iE force trace underneath iE EMG trace. Note that the period between extensor bursts on the contralateral side is temporarily reduced because of the ventral root stimulation or the triceps surae vibration. The resulting contralateral rhythm resetting paralleled the resetting of the ipsilateral rhythm. Force calibration in (A) = 6.5 kg.

### (1) *Delay of flexor burst by vibration of the triceps surae*

In previous sections evidence was presented indicating that afferent activity from the triceps surae may prevent the generation of a rhythmic flexor burst during locomotion. The same effect was obtained by stimulating large cutaneous afferents from the distal hindlimb, but in addition the stimulation of cutaneous afferents during the extension phase yielded a prolongation of the step cycle due to a delay in the onset of the flexor burst (Chapter 2). Such flexor burst delaying effects were also found in the present experiments when short periods of vibration were applied during the locomotory process. As shown in Fig. 32 (top) triceps surae vibration started in the extension phase often caused the following flexor burst to be delayed rather than suppressed. This caused the step cycle during which vibration was started to be prolonged from 720 msec to 840 msec. The delayed flexor burst was unchanged in duration or only slightly shorter than the flexor burst preceding the application of vibration and the small changes in duration of the delayed flexor burst could not account for the prolongations of the step cycle. The step cycle following the prolonged step cycle had a duration (740 msec) comparable to the duration of the step cycles preceding vibration (720 msec).

By taking a number of consecutive vibration trials, a plot as shown in Fig. 32 (bottom) could be made. The duration of the step cycle during which vibration was started was compared to the duration of the preceding step cycle and the difference was expressed as a percentage of the preceding step cycle duration. The resulting

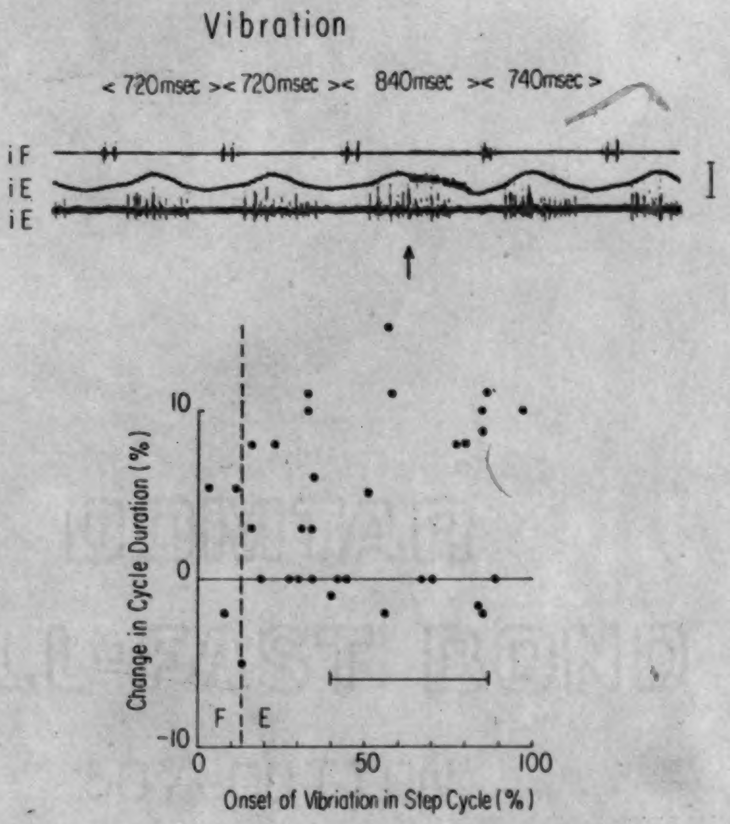


Fig. 32. Delay of the rhythmic bursts of activity in the pretibial flexors due to vibration of the triceps surae.

Top: Filmed example, showing the prolongation of the step cycle (time between onset of consecutive flexor bursts) when a 400 msec period of vibration at 64 Hz was started (arrow) in the extension phase. Step cycle measurements on top of record (720, 720, 940, 740 msec) were taken with a resolution of 1 mm = 20 msec (film speed = 5 cm/sec). Force calibration = 3 kg. iF, iE and cE as in Fig. 29.

Bottom: Plot of change in step cycle duration (duration of step cycle in which vibration is started minus the duration of the just preceding step cycle) versus time of onset of vibration within step cycle, both normalized with respect to the duration of the step cycle just preceding the application of the vibratory stimulus. These preceding step cycles averaged 743 msec in duration and the flexor burst took up 13% of that duration (vertical dashed line between F = flexion and E = extension). Bar on lower half of plot indicates the duration of the vibratory stimulus relative to the average step cycle duration.

"change in cycle duration" was plotted versus the time between the onset of the step cycle and the onset of vibration (normalized with respect to the preceding step cycle). The results indicated that there was a tendency for the step cycles to be prolonged by the application of triceps surae vibration. However, this tendency was not very strong, as can be judged from the small amount of prolongation (on the average 4.5%) as compared to the normal variability of the step cycles (S.D. of step cycles just preceding vibration was 5% of the average duration of these preceding cycles). In another cat, the prolongations were calculated to average 9% (N=12), which was statistically significant at the 0.005 level (step cycles preceding the step cycle with vibration differed from the step cycles following the vibration trial by only 1%). The large variability in the responses to vibration was maybe related to the fact that the flexor burst delaying effects were very critically dependent both on the amplitude of vibration and on the amount of background stretch of the vibrated muscle. Unfortunately, our experimental conditions did not allow us to make a systematic study of these parameters in the 4 cats (out of 5) in which flexor burst delaying effects were observed.

It should be emphasized that these vibration experiments were not designed to answer the question of which type of muscle afferents plays a role in suppressing or delaying the flexor burst. Our vibratory stimulus probably activated Ia spindle afferents from the triceps surae, but other types of muscle afferents were likely to be activated as well because of the evoked muscle contraction.

### C. *Nerve stimulation*

#### (1) *Acute experiments*

In 8 cats the proximal end of the cut nerves to the left gastrocnemius-soleus and/or medial gastrocnemius were stimulated periodically with 100 msec trains of 6 pulses at 1.3 and 1.7 x T. The purpose of these experiments was to see if the above described results obtained with natural activation of triceps surae afferents could be confirmed using direct electrical stimulation of the large triceps surae afferents.

Stimulation of the triceps surae nerves just before or during the occurrence of the flexor burst caused a reduction in the amplitude and duration of the flexor EMG burst, as expected on the basis of reciprocal inhibition, but there was no reproducible change in the rhythmic appearances of these flexor bursts even when the gastrocnemius-soleus and the medial gastrocnemius nerves were stimulated together.

Only when stimuli above group I strength were applied was there evidence for interference with the locomotory rhythm (Fig. 33). Stimulation of the cut medial gastrocnemius nerve at FRA strength (33 V while T not measured but estimated at around 3 V) caused the following flexor burst to start later than expected, provided the stimuli were applied during the extension phase (middle and bottom example on top of Fig. 33). This resulted in a prolongation of the step cycle duration, measured as usual from the beginning of one flexor burst until the onset of the next one (plot on bottom of Fig. 33). However, the delay of the onset of the next flexor burst did not

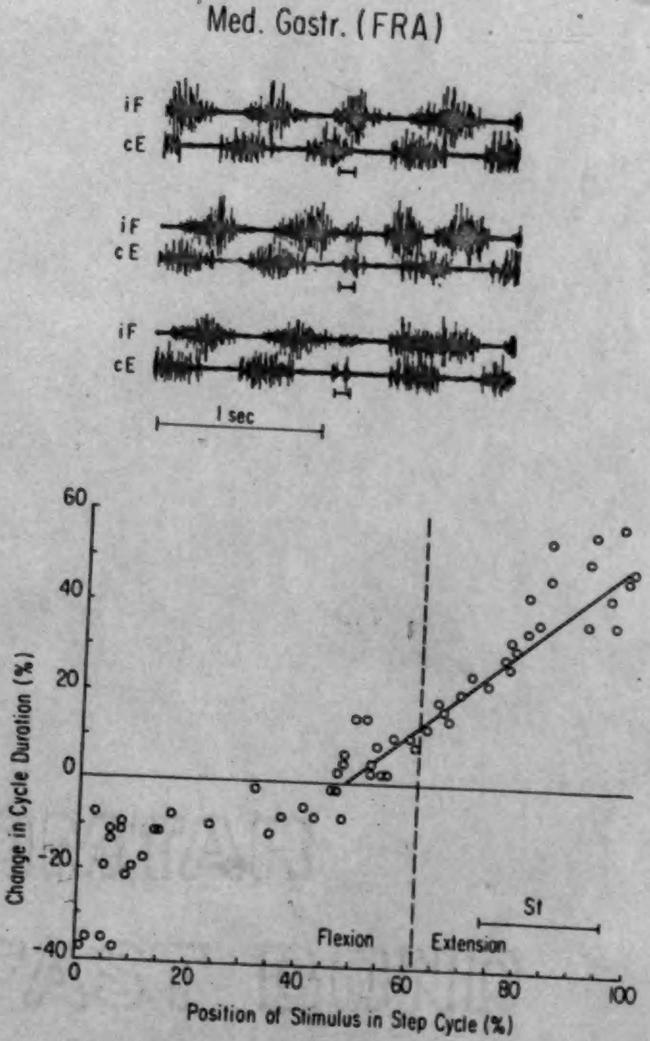


Fig. 33. Effect of stimulation of the medial gastrocnemius nerve on the locomotory output. iF = ipsilateral pretibial flexors; cE = contralateral triceps surae; St = 100 msec train of 0.05 msec pulses at 60 Hz (33 V). Note that the later the stimuli were given in the extension phase, the more the following flexor burst was delayed, hence leading to an almost linear increase in the step cycle duration.

necessarily correspond with an equal amount of rhythm resetting, indicating that part of the delay of the flexor burst may simply have been due to direct effects on flexor motoneurons. Nevertheless, there is no doubt that the stimulation also affected the rhythm generating system since changes in rhythm were not only observed ipsilaterally but also contralaterally (Fig. 33 top).

### (2) *Chronic experiments*

The relative ineffectivity of triceps surae nerve stimulation in producing effects on the locomotory rhythm was confirmed in a normal cat with a cuff implanted around the lateral gastrocnemius nerve and the sciatic nerve. Even when stimuli of 2.5 or 3 x T (6 pulses of 0.01 msec at 60 Hz) were applied during the periods of walking on the treadmill, no consistent effects were produced on the duration of the phase during which stimulation was started (in the two experiments done so far).

It thus seems that low intensity stimulation of muscle nerves is less effective in changing the duration of step cycle phases than low intensity stimulation of cutaneous nerves (Chapter 2). However, more experiments are needed to substantiate the latter point.

## 3.4 *Discussion*

The data presented above provide evidence that afferent activity from the triceps surae can inhibit the central system responsible for the generation of rhythmic bursts of activity in the flexors during locomotion. This central system must be at a premotoneuronal level since afferent activity from the triceps surae caused the rhythmic flexor burst either to disappear in an all-or-none fashion or to be delayed. Neither one of these effects can simply be explained in terms of excitability changes at the motoneuronal level since there is no evidence that motoneuronal pools have synchronizing properties on their own. Rather, one has to invoke the existence of interneuronal centers which form the basis for the generation of bursts of activity in flexors or extensors during locomotion. As in the previous chapter, such centers will be called "Flexor Half-Center" (FHC) and "Extensor Half-Center" (EHC).

The data thus support the idea that triceps surae afferents make inhibitory connections with the FHC. The functional significance of this connection is much the same as for the inhibitory connection between large cutaneous afferents and the FHC (Chapter 2). The need for an animal to be supported by some of its limbs during walking at moderate speeds calls for a system which allows for the flexion of a limb to occur only when sufficient support is provided by the other limbs. Hence it would be extremely useful for the walking animal to have in each limb an automatic in-built mechanism for the triggering of flexion after a signal is given that the limb in question is no longer loaded and no longer needed to provide support for the animal. A decline in afferent activity from the extensor muscles may constitute such a signal and consequently, if such a decline is prevented, for example by preventing the limb from fully extending, then the FHC will fail to be activated and stepping will be abolished in the restrained limb (see Orlovsky and Feldman, 1972; Grillner, 1975).

At present, the exact location of the inhibitory pathway from triceps surae afferents to the FHC remains unknown. Supraspinal centers are probably not essential since blocking of the rhythm of a single limb was observed in the chronic spinal dog or cat (Sherrington, 1906; Grillner, 1975). At the spinal cord level a system of interneurons exists which is activated by intravenous injection of DOPA and which is likely to be identical to the FHC (Jankowska, 1967b). In close proximity and partly overlapping with this system, many interneurons are found which receive connections from both muscle and skin afferents (Szentágothai, 1967; Eccles *et al.*, 1956) and some of which have no direct projections to motoneurons (Hultborn *et al.*, 1971; Hongo *et al.*, 1972). The latter interneurons, located within the intermediate nucleus, may well form the anatomical basis of the presently described effects. The convergence of proprioceptive and exteroceptive afferent input onto these interneurons may explain why the flexor burst suppressing or delaying effects were also obtained in the experiments described in Chapter 2. In this respect it is of interest that it was recently found that an extensive convergence exists between large cutaneous afferents and Ib muscle afferents (Lundberg *et al.*, 1975). Moreover, in a study on spinal interneurons presumably belonging to the locomotory centers, Edgerton *et al.* (1976) found that activity in some of these interneurons was affected by volleys in cutaneous nerves, while others responded to group I volleys in muscle nerves.

Our negative results after group I stimulation of the triceps surae nerves do not exclude the possibility of an inhibitory connection between group I muscle afferents and the FHC, since our failure may have simply been due to an unfortunate choice of stimulus parameters. Further experiments are needed to clarify which type of muscle afferents are inhibitory to the FHC.

# CHAPTER 4. THE ROLE OF JOINT AFFERENTS IN LOCOMOTION

## 4.1 *Introduction*

Virtually nothing is known about the role of joint afferents in locomotion. For the knee, Freeman and Wyke (1966) reported that cats had great difficulty in walking along a narrow bar when their posterior and medial articular nerves were cut. However, when Lindström and Norsell (1971) repeated these experiments, they were unable to detect any deficits at all.

Joint afferents from the hip may be of greater importance, however, since it is thought that joint afferents from the hip (or afferents from hip muscles) may provide the "hip afferent signal" which may play a dominant role in reflexly inducing the swing phase at the end of stance (Rossignol *et al.*, 1975). The latter idea was originally based on the observations of Shik and Orlovsky (1965), who found that raising the anterior or posterior part of a normal dog walking on a treadmill induces a prolongation of both the stance phase and the step cycle of the raised limbs. They reasoned that the prolongation of the stance phase in the raised limbs may have been correlated with an unchanged angular excursion of the hip and shoulder and hence they concluded that "the initiation of the transfer phase is determined by the onset of threshold extension of the limb, i.e. at definite values of the joint angles". Further support for this "threshold extension" hypothesis was provided by the work of Grillner and his colleagues (Grillner, 1975), who found that when the hindlimb of a spinal cat is prevented from fully extending during the stance phase, a maintained extensor burst is evoked which only ceases by bringing the femur more caudally to an angle equivalent to the hip angle at which the swing phase is normally initiated.

However, the use of such rigid criterion as a "threshold extension" at the hip seems highly improbable since the excursion of the hip per se does not seem to give the animal much information about the appropriateness of ending support and starting flexion (see also Wetzel and Stuart, 1976). More likely the initiation of the swing is determined by a combination of factors, some of which are related to a fall in activity in exteroceptive and proprioceptive afferents which are normally activated during the stance phase (see Chapters 2 and 3).

In the work to be presented in this chapter, an answer was sought to the following questions: (i) does the cat always initiate the swing phase when the hip reaches a "threshold angle"; and (ii) are the hip joint afferents crucial for the initiation of the swing phase and for locomotion in general? Three techniques were used:

1) a repeat of the Shik and Orlovsky (1965) experiments in mesencephalic cats;
2) observations on normal cats walking while crouched or while loaded;
3) hip joint denervation in the normal cat.

## 4.2 *Methods and results*

### 4.2.1 *Lifted mesencephalic cats*

In two mesencephalic cats the posterior body part was raised and the effects on the step cycle examined. It was found that lifting the animal did not change the step cycle duration by more than 3%, although the duration of the EMG burst in the ankle extensors was reduced to 40% of its normal value. These results differ from the results of Shik and Orlovsky (1965) as outlined above. Possibly this difference is due to the fact that the Russian workers used normal dogs with suspended forelimbs while the present experiments were done on mesencephalic cats which could step on the treadmill with their forelimbs. Nevertheless, the present findings seem to warrant a cautious reappraisal of the conclusions of Shik and Orlovsky (1965).

### 4.2.2 *Behavioral analysis on normal cats*

One way to test whether there are features of the step which are kept constant during locomotion (such as the hip angle at the initiation of the swing) is to put severe constraints on the locomotory behavior and see if these "constant" features remain even in the most taxing circumstances. Two experimental situations were tested, namely crouching and loading.

#### A. *Crouching*

Two adult cats were trained to walk on a treadmill with an adjustable ceiling. First the cats were trained with positive reinforcement techniques so that they learned to walk on a treadmill without showing a crouching posture. Then the ceiling of the cage over the treadmill was lowered so that the cats were forced to crouch. Training was considered satisfactory when the cats could maintain a constant speed of locomotion with the treadmill belt moving at 1.25 m/sec. One cat weighing 2.6 kg could perform the task when the ceiling was lowered so as to leave only 13 cm distance between the belt and the ceiling. The other cat (4.6 kg) could still maintain a steady gait when forced to crouch under a ceiling placed 20 cm above the belt. The left hindlimb of both cats were shaved and dots were painted on the skin above bony landmarks such as described by Goslow *et al.* (1973). The cats were filmed with high speed cinematography (64 frames/sec). The filmed material was examined for sequences where the position of the cat was constant in relation to the treadmill cage (indicating that the cat walked exactly at the speed of the treadmill belt). Such sequences were analyzed frame by frame for joint angle excursions of the left hindlimb. Since some slippage of the skin over bony landmarks during locomotion is unavoidable, it was usually found more reliable to measure the joint angles by drawing tangent lines on the body surface rather than to use the painted dots on the skin. Hence, the hip angle was measured as the angle between the line tangent to the lower back of the cat and the line tangent to the frontal surface of the thigh. Similarly, the knee and ankle angles were measured by drawing lines tangent to the frontal surface of the thigh and tha shank and to the dorsal surface of the shank and the foot respectively. This method yields very reproducible results as long as care is taken to keep the contact points of the tangent lines constant from frame to frame.

When studying the joint excursions during the stepping movements, it became obvious that the hip behaved in a very peculiar way during forced crouch. In contrast to the knee and ankle, which were always held more flexed during the step cycle of the forced crouch than during the normal step cycle, the hip was held less flexed during most of the same period. At the end of the stance phase of the "crouch" step cycle the hip was extended up to 30° more than normal while the hip position at the beginning of the stance phase was the same in the "crouch" and the "normal" situation (Fig. 34). Such hyperextensions of the hip at the end of the stance phase were a constant finding for crouching under a 20 cm high ceiling but were less obvious when the crouching was less pronounced due to higher ceilings.

Inspection of the evolution of the excursions at the other joints (knee, ankle) also revealed some interesting findings. Fig. 35 compares the joint angles of the hip, knee and ankle for cats walking normally and in a crouched position. The durations of the step cycle, swing and stance phase were the same whether or not the cats assumed a crouching position. However, the joint excursions made within these periods of constant duration were markedly different for the two experimental situations. The onset of the first extension phase, as judged from the first increase in joint angle, was clearly delayed for the knee joint and even more so for

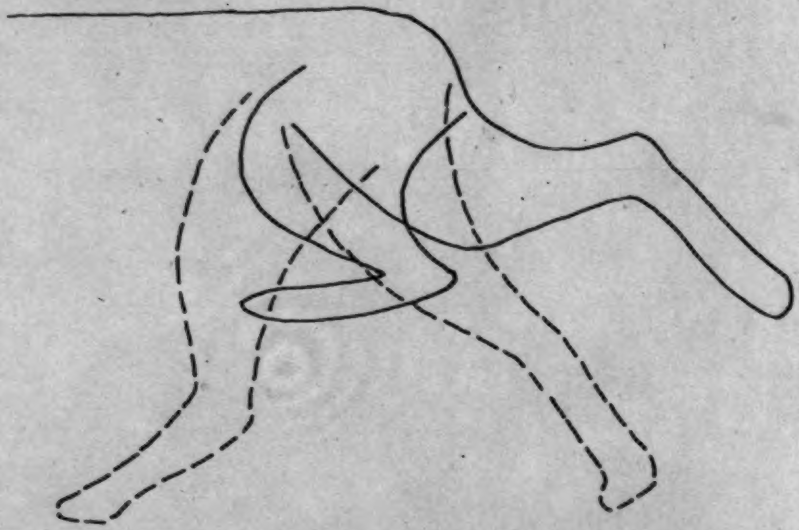


Fig. 34. Changes in the position of the left hindlimb at the beginning and ending of the stance phase when the cat was forced to crouch (full lines) or walked normally (dashed lines) on a treadmill. Speed of belt: 1.25 m/sec. Drawing was made by taking the first and last frame of foot contact during the stance and superimposing those frames for four subsequent step cycles. Note the hyperextension of the hip at the end of the stance phase when the animal was crouching.

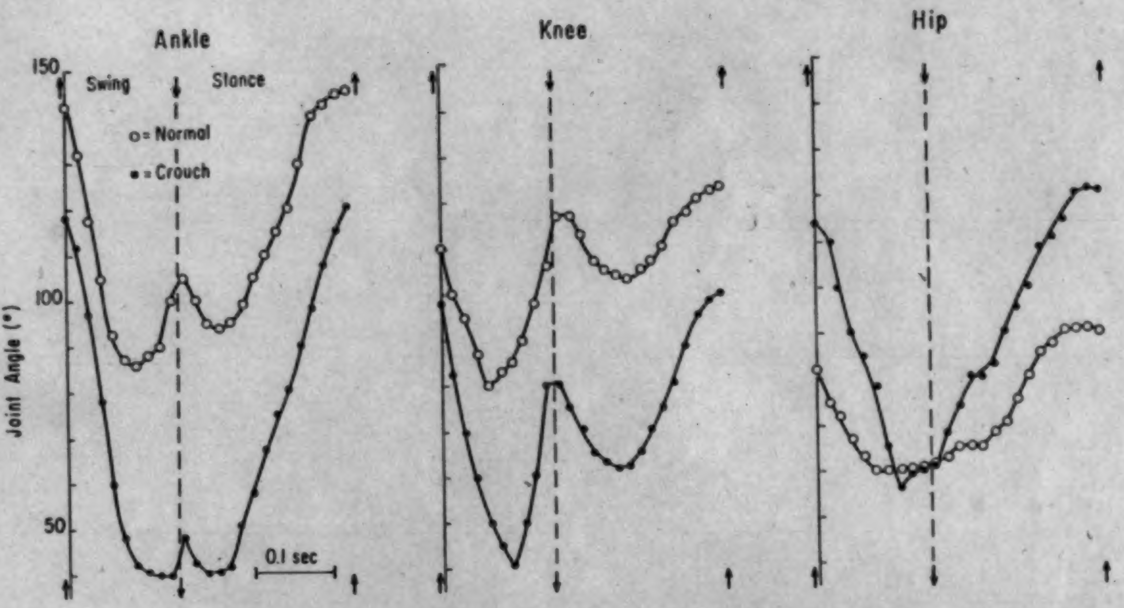


Fig. 35. Comparison of joint angle excursions of a cat which either walked normally on a treadmill (open circles) or was forced to crouch under a ceiling placed 13 cm above the treadmill belt (closed circles). Two representative step cycles of the same duration were chosen for comparison. Speed of belt: 1.25 m/sec. Onset of swing and stance indicated by downward and upward arrows respectively.

the ankle joint when the animals were forced to crouch. The interlimb coordination in the crouch situation was characterized by a shorter time interval between the onset of the first extension phase at the knee of the hindlimb and the moment of forelimb lift-off (34 msec versus 64 msec with S.D. of 9 and 19 respectively, N=20). Similar results were obtained by Wetzel *et al.* (1975) when describing the kinematic differences between cats stepping on a treadmill and cats walking over ground. In cats walking on a treadmill 67% of the duration of the swing phase was occupied by flexion while this was only 46% for the cats walking over ground, the total duration of the swing phase being identical for the two situations. In addition, they found that on the treadmill the knee began to extend some 27 - 41 msec before the ankle at the transition from flexion to extension during the swing phase. Interlimb coordination was also affected since the interval between the onset of extension during the swing phase of the hindlimb and onset of flexion at the beginning of the swing phase in the forelimb was smaller for cats walking on a treadmill. Wetzel *et al.* (1975) attributed these differences to the more crouched position maintained by their cats during treadmill locomotion. The crouching was thought to be due to their training method based on avoidance of air jets although a minimal amount of crouching was observed even when positive reinforcement was used.

In summary, a comparison of joint angle excursions in cats walking on a treadmill with adjustable ceiling revealed that a crouching posture can account for some of the changes previously observed when treadmill walking was compared to overground walking, thus confirming the hypothesis of Wetzel *et al.* (1975). The additional finding that hip extension at the end of stance can be some 30° larger during walking in a crouching posture casts some doubt on the concept that a threshold extension at the hip is used as a trigger signal for the initiation of the swing phase.

### B. *Loading*

The same cats used in the crouch experiments were filmed in an upright position but with a load of 500 grams (Pb) on their back. As seen in Fig. 36, this had little effect on the position of the hip at the beginning and at the end of the stance phase. The main changes observed were in the knee and ankle joints, which were held more flexed than normal.

Loading of the cats had also no effect on the duration of the step cycle, as was also observed when mesencephalic cats were loaded with 500 grams. These findings are in agreement with Orlovsky *et al.* (1966), who found that normal dogs pulling a load or walking uphill on a treadmill show no appreciable change in step cycle duration.

### 4.2.3 *Hip joint denervation*

To evaluate the contribution of hip joint afferents to the execution of walking movements, a bilateral hip joint denervation was done in a cat which was previously trained for treadmill locomotion. The hip joint of the cat is supplied by three nerves, which Dee (1969) designated as the medial articular nerve, the nerve to the ligamentum capitis femoris and the posterior articular nerve. These nerves join

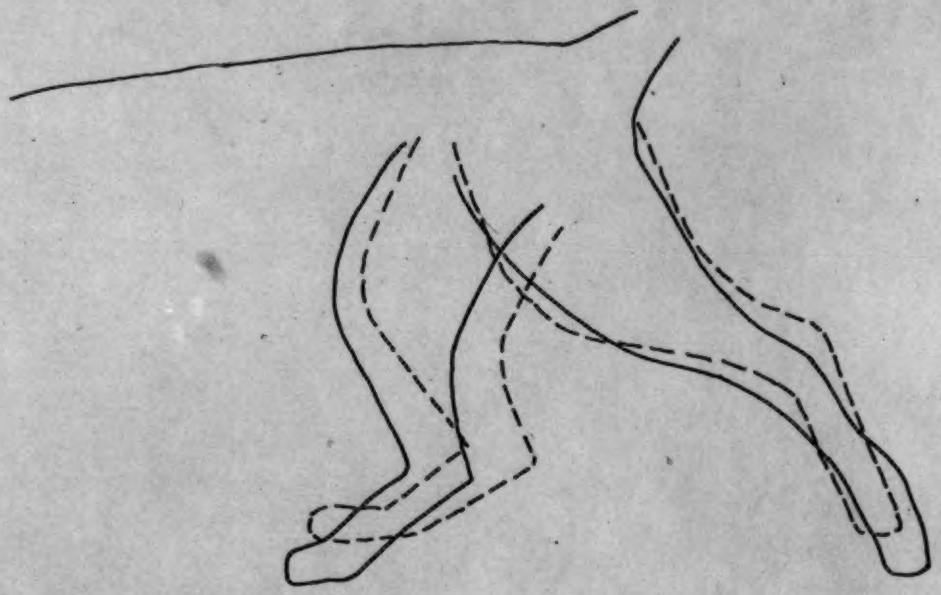


Fig. 36. Differences in the position of the left hindlimb at the beginning and ending of the stance phase when the cat was loaded with 500 grams on its back (dashed lines) or when walking normally (full lines). Superposition of four frames for each limb position. Right hindlimb not shown. Note the more crouching posture when the animal was loaded.

muscle nerves supplying the adductor muscles, the pectineus, the obturator externus and the quadratus femoris. Denervation of the hip joint could only be achieved by denervating these muscles as well. As expected, the cat lost most of its adduction capabilities post-operatively and this made it impossible for the cat to walk, especially when put on a slippery surface. However, the ataxia gradually disappeared and on the third day following the operation the cat was already able to walk without any noticeable deficit. However, the cat had great difficulty in walking when it was forced to do so in a crouching position due to an artificial ceiling over the treadmill. When crouching, the hindlimbs frequently yielded excessively at the end of the stance phase. This deficit may be attributed to denervation of either the hip joint or the adductor muscles or both.

The observation that bilateral hip denervation does not produce major deficits in the pattern of locomotion under normal circumstances indicates that joint afferents from the hip are not indispensable for normal locomotion. Further support for this conclusion is provided by the observation that patients with total hip replacement can walk normally. In fact, such patients even retain the ability to detect hip joint position (Grigg *et al.*, 1973). No descriptions of gait deficits after hip joint deafferentation in the cat are available to our knowledge.

## 4.3 *Discussion*

The present data on the raised mesencephalic cats and on the crouching normal cats do not provide positive or negative evidence for the idea of the "hip afferent signal" but they do provide some arguments against the idea that the initiation of the swing phase is always reflexly triggered when the hip reaches a "threshold" angle. Clearly, one has to be very cautious when defining a particular feature of the step cycle as a "standard" feature. Most kinematic studies are done under standardized conditions and it is thus not surprising that "standard" features are discovered. A good example is the constancy of the swing phase duration. Although it is true that animals walking over even surfaces at different speeds tend to have a standart duration swing phase, this does not imply that the swing phase is always constant under all circumstances (for examples see Chapter 2). The same is true for the standard hip angle at the end of stance. It would be a great disadvantage for any animal if it had to rely on a particular joint angle excursion for the initiation of the swing phase since this would imply a dramatic limitation in the flexibility of its locomotory behavior. Rather, one would expect that such important decisions as the ending of the support phase would be determined by a number of factors related to skin, muscle and joint afferents from different sources as was amply discussed in previous chapters.

# CHAPTER 5. FUTURE WORK

From the previous chapters it is clear that we are only starting to understand the role of afferents in walking. In this chapter a few areas will be outlined in which future work is needed to further clarify issues raised in the course of this thesis.

## 5.1 *Identification of the flexor half-center*

The hypothesis that different groups of cutaneous afferents make different types of connections with the flexor half-center needs further exploration using intracellular techniques. Perhaps the first step should be the identification of the half-center itself. In the acute spinal cat treated with DOPA or Clonidine, the flexor half-center probably corresponds to a group of interneurons responsible for the generation of long-latency, long-lasting discharges in the ipsilateral flexor motoneurons after stimulation of the sural nerve (Jankowska *et al.*, 1967a, b). Similarly, such late flexor discharges can be evoked in the mesencephalic cat after brainstem stimulation (Grillner and Shik, 1973).

The appearance of the late flexor discharge is thus closely linked to the activation of the spinal centers for locomotion and one would expect therefore to find such late discharges also in the premammillary cat showing spontaneous locomotion. Preliminary data presented in Fig. 37 show that such late discharges may indeed be found in the premammillary cat. In one experiment, sural nerve stimulation yielded a small reflex contraction of the triceps surae

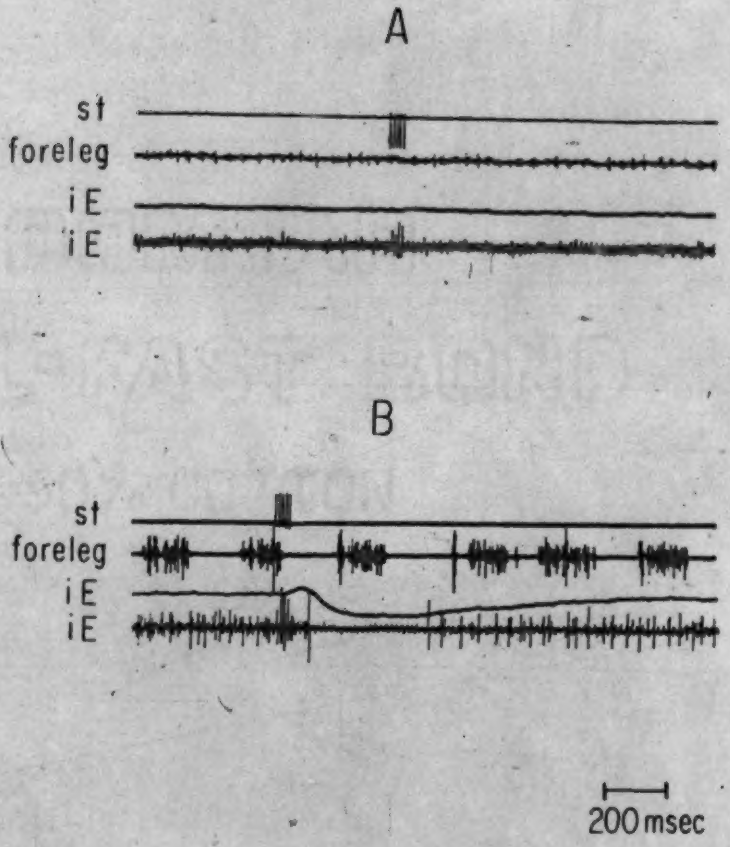


Fig. 37. Forelimb stepping as a method to release the late long-lasting triceps surae inhibition following tetanic sural nerve stimulation. (A) Resting premammillary cat showing very small reflex contraction in the ipsilateral triceps surae (iE = force and EMG) following sural nerve stimulation (St). (B) The appearance of rhythmic alternating movements in the forelimbs is correlated with the appearance of a long-lasting triceps surae relaxation when the same stimuli are applied.

in the resting animal (Fig. 37A) but the same stimuli applied in a period during which the forelimbs moved and the hindlimbs stayed immobilized evoked a short contraction followed by a long-lasting relaxation of the triceps surae. Unfortunately, no flexor EMG was recorded but it seems highly likely that the long extensor inhibition was correlated with a long flexor excitation. Nevertheless, the data of Fig. 37 show that it may be possible to demonstrate the late flexor discharge, thought to arise from the flexor half-center, in the spontaneously walking premammillary cat, hence providing a link with the data on spinal and mesencephalic cats and hence providing the possibility of exploring intracellularly the flexor half-center in the curarized premammillary cat.

The development of such a preparation should allow one to follow up on the very exciting results recently obtained by Edgerton *et al.* (1976), who recorded from interneurons which are rhythmically active in the spinal curarized cat injected with DOPA and Nialamide. They found that stimulation of the ipsilateral peroneal nerve with a short train of 3 pulses at 20 x T could produce a rebound of activity in an interneuron which had its main activity together with the activity in ipsilateral flexor motoneurons. Since the latter interneuron may well have been part of the flexor half-center, their finding constitutes the first intracellular evidence for the rebound of the flexor half-center following peripheral nerve stimulation. By stimulating cutaneous nerves at different moments in the time course of activity of such flexor half-center interneurons, it should be possible to demonstrate at an intracellular level the interactions between large cutaneous afferents and the flexor half-center as proposed in Chapter 2.

## 5.2 *Late reflex responses in premammillary cats*

In recent years, advances have been made in our understanding of late reflexes, presumably involving the motor cortex (Tatton and Lee, 1975; Murphy *et al.*, 1974). Long-loop reflexes, not involving the motor cortex, have also been explored but only one such reflex has been well documented, namely the spino-bulbo-spinal reflex (S.B.S. reflex: Shimamura *et al.*, 1965), which is excitatory to flexors. So far there is no evidence for long-loop reflexes to extensors in decerebrate animals.

However, in the present study it was noted at several occasions that late reflex waves could be observed in the triceps surae after stimulation of the tibial or peroneal nerve in premammillary cats. Fig. 38 shows an example of an experiment in which the common peroneal nerve was stimulated in a cuff electrode while the EMG response of the triceps surae was recorded. Two late waves were detected by averaging the EMG response in the triceps surae (N=200). Both late waves increased in amplitude when the stimulus intensity was increased from 2 x T to 2.8 x T. Interestingly, the onset of the last late wave shifted forward in time from 63 msec to 59 msec when the stimulus intensity was increased. The origin of these responses in the premammillary cat is uncertain, but further experiments along this line (with decerebellectomy, variations in triceps surae stretch, selective lesions at the spinal and supra-

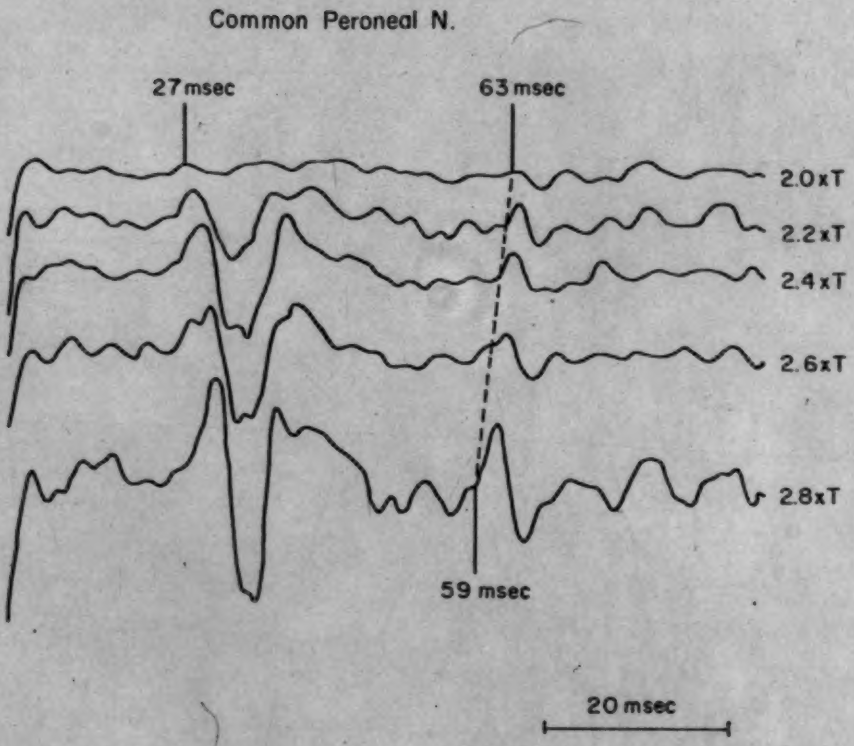


Fig. 38. Polysynaptic late reflex responses in the triceps surae following stimulation of the intact common peroneal nerve. Each trace is the computer average of 200 unrectified EMG responses. Sample interval = 0.2 msec. To accentuate the late waves, a delay of 8 msec was introduced to eliminate the large early response, the end of which is just visible at the beginning of each record. Digital filtering allowed the averaging of subsequent groups of seven bins within the total of 512 bins for each record. Resting premammillary cat.

level) will undoubtedly be of great interest.

### 5.3 *Phasic disturbances of the gait*

A third major area for future research is for behavioral experiments on normal cats in order to test some of the hypotheses proposed in this thesis. If muscle and skin receptors do play a role in the regulation of the stance phase, then one should be able to demonstrate this in normal cats put in a situation where these receptors are naturally stimulated during locomotion. Such a situation can be created in a number of ways. One I have used is to put cats in a special cage and let them step on a trap door which moves upwards following foot contact, hence introducing a phasic perturbation of the stance phase. The upward movement of the trapdoor was triggered by a short-circuit produced by contact between the copper covering of the trapdoor and the copper shoe put around the foot of the cat's left hindlimb. The experimenter could switch off the short-circuiting system activating the trap door so that not all foot contacts were followed by trap door movement. A small light indicated the moment of foot contact so that the delay between foot contact and upwards movement of the trap door could be calculated from the frame-to-frame analysis of the high speed cinematographic movies made of the experiments.

The preliminary results showed that both cats reacted to the upwards movement of the trapdoor by hyperextension of the whole limb at the end of the stance phase. However, simple cinematographic analysis did not allow us to decide whether this was a true reflex reaction or simply a mechanical perturbation. Normal cats with implanted EMG electrodes will probably enable us to determine the true reflex nature of this response.

Finally, gait perturbation experiments should be done on normal human subjects. What reflex adjustments are made when certain peripheral nerves are stimulated in humans walking on a treadmill? Preliminary experiments by Dr. Herman indicate that sural nerve stimulation indeed promotes extension when given during the stance of normal walking subjects (personal communication). Or what are the reflex responses of a normal subject, presented with an even surface on which to walk, then blind-folded and allowed to walk over the same surface which now has unexpected elevations or holes?

# CHAPTER 6. SUMMARY

Afferent input is not required for the production of stepping movements but is essential for the adjustment of the step to external requirements. The way in which afferent input modulates stepping was investigated by introducing short- and long-term changes in afferent input during walking of mesencephalic, premammillary and normal cats and by examining the resulting changes in EMG activity and joint angle excursions. The effects of three types of afferents were studied.

1. *Skin Afferents.* Cutaneous afferents arising from the distal hindlimb have been suspected to play an important role in locomotion but evidence was lacking. In the present work it was found that electrical stimulation of the skin of the distal hindlimb or of the skin afferents innervating this area increased the duration and/or amplitude of the EMG activity in the ankle extensors when the stimulation was applied during the stance phase of spontaneously walking premammillary cats. When applied during the flexion phase, the stimuli either prolonged or shortened the ongoing burst of activity in the ankle flexors, depending on the timing and intensity of the stimuli. Stimulation of the tibial nerve at 2.5 x threshold yielded a flexor burst prolongation when stimuli were applied at the beginning of the flexion phase. However, when applied at the end of flexion the same stimuli caused a flexor burst shortening and a premature onset of extension. Stronger stimuli given during the flexion phase evoked a

flexor burst prolongation regardless of the time of stimulus application. Very strong shocks, inducing a flexor reflex, evoked a bilateral resetting of the rhythm except if stimulation was applied just prior to or at the beginning of the ipsilateral flexion phase.

In normal cats, only moderate stimulation was used on the tibial, peroneal and sural nerves. Low threshold stimulation during the stance increased the ongoing extensor activity in the ankle extensors. When applied during the flexion phase, the stimuli caused a prolongation of the flexion or, more rarely, a shortening.

The results were interpreted in function of reflex actions of large and small cutaneous afferents. Activity in large afferents, presumably arising from mechanoreceptors in the distal hindlimb, may assist the animal in supporting itself by causing increased extensor activity during stance. Activity in the large cutaneous afferents at the end of the flexion phase may cause a premature onset of extension so that the limb is ready to take up the support of the body if the foot touches an unexpected elevation at the end of the flexion phase. On the other hand, activity in smaller afferents, related to the detection of noxious stimuli, may cause a shortening or temporary interruption of extension during stance. During flexion the same noxious stimuli may cause a prolongation of the flexion phase so that in all cases contact with the noxious stimuli is avoided.

2. *Muscle Afferents.* Extensor muscle receptors are active during locomotion but little is known about their role in the regulation of the step cycle. In this study it was found that the rhythmic contractions of the ankle flexors and extensors in the fixed hindlimb

of premammillary cats can be made to disappear by stretching the ankle extensors in the fixed hindlimb or by making the triceps surae to contract by ventral root stimulation or by triceps surae vibration. A second method for blocking the rhythm selectively in a single limb consisted of leaving the left limb free except for the ankle joint which was clamped in a flexed position. In all cases the rhythmic flexor burst was seen to disappear in an all-or-none fashion. In addition, it was found that short periods of vibration of the triceps surae could lead to a delay in the onset of the flexor burst. It is suggested that unloading of the ankle extensors is required for the release of activity in the central system involved in the generation of the flexion phase in walking.

3. *Joint Afferents.* Afferents from the hip joint may signal when the hip passes through a "threshold extension" at the end of stance and this signal may reflexly trigger the flexion phase. Evidence against this idea of "threshold extension trigger" was obtained when normal cats were filmed while forced to crouch on a treadmill. Hip extension at the end of stance could be up to 30° larger than under normal circumstances, indicating that the initiation of the flexion phase in normal cats is not always related to a standard hip extension. Furthermore, hip joint afferents were shown not to be essential for walking since bilateral denervation of the hip joint did not produce permanent gait deficits.

It was concluded that the transition from flexion to extension and from extension to flexion is profoundly affected by afferent input from exteroceptive and proprioceptive sources although the spinal cord itself can produce such switching in the absence of sensory feedback. Furthermore, reflex actions from these afferent sources may reinforce the ongoing activity either through "private" pathways to motoneurones or through pathways to interneuronal half-centers, involved in the generation of generalized flexion or extension in individual limbs.

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